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THE
BIOLOGICAL BULLETIN

PUBLISHED BY
THE MARINE BIOLOGICAL LABORATORY

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PUBLISHED BY THE MARINE BIOLOGICAL LABORATORY

THE MARINE BIOLOGICAL LABORATORY

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II. ACT OF INCORPORATION

No. 3170

COMMONWEALTH OF MASSACHUSETTS

Be It Known, That whereas Alpheus Hyatt, William Sanford Stevens, William T. Sedgwick, Edward G. Gardiner, Susan Minns, Charles Sedgwick Minot, Samuel Wells, William G. Farlow, Anna D. Phillips, and B. H. Van Vleck have associated themselves with the intention of forming a Corporation under the name of the Marine Biological Laboratory, for the purpose of establishing and maintaining a laboratory or station for scientific study and investigation, and a school for instruction in biology and natural history, and have complied with the provisions of the statutes of this Commonwealth in such case made and provided, as appears from the certificate of the President, Treasurer, and Trustees of said Corporation, duly approved by the Commissioner of Corporations, and recorded in this office;

Now, therefore, I, HENRY B. PIERCE, Secretary of the Commonwealth of Massachusetts, *do hereby certify* that said A. Hyatt, W. S. Stevens, W. T. Sedgwick, E. G. Gardiner, S. Minns, C. S. Minot, S. Wells, W. G. Farlow, A. D. Phillips, and B. H. Van Vleck, their associates and successors, are legally organized and established as, and are hereby made, an existing Corporation, under the name of the MARINE BIOLOGICAL LABORATORY, with the powers, rights, and privileges, and subject to the limitations, duties, and restrictions, which by law appertain thereto.

Witness my official signature hereunto subscribed, and the seal of the Commonwealth of Massachusetts hereunto affixed, this twentieth day of March, in the year of our Lord One Thousand Eight Hundred and Eighty-Eight.

[SEAL]

HENRY B. PIERCE,
Secretary of the Commonwealth.

III. BY-LAWS OF THE CORPORATION OF THE MARINE BIOLOGICAL LABORATORY

I. The members of the Corporation shall consist of persons elected by the Board of Trustees.

II. The officers of the Corporation shall consist of a President, Vice President, Director, Treasurer, and Clerk.

III. The Annual Meeting of the members shall be held on the second Tuesday in August in each year, at the Laboratory in Woods Hole, Massachusetts, at 11:30 A.M., and at such meeting the members shall choose by ballot a Treasurer and a Clerk to serve one year, and eight Trustees to serve four years, and shall transact such other business as may properly come before the meeting. Special meetings of the members may be called by the Trustees to be held at such time and place as may be designated.

IV. Twenty-five members shall constitute a quorum at any meeting.

V. Any member in good standing may vote at any meeting, either in person or by proxy duly executed.

VI. Inasmuch as the time and place of the Annual Meeting of members are fixed by these By-laws, no notice of the Annual Meeting need be given. Notice of any special meeting of members, however, shall be given by the Clerk by mailing notice of the time and place and purpose of such meeting, at least fifteen (15) days before such meeting, to each member at his or her address as shown on the records of the Corporation.

VII. The Annual Meeting of the Trustees shall be held on the second Tuesday in August in each year, at the Laboratory in Woods Hole, Mass., at 10 A.M. Special meetings of the Trustees shall be called by the President, or by any seven Trustees, to be held at such time and place as may be designated, and the Secretary shall give notice thereof by written or printed notice, mailed to each Trustee at his address as shown on the records of the Corporation, at least one (1) week before the meeting. At such special meeting only matters stated in the notice shall be considered. Seven Trustees of those eligible to vote shall constitute a quorum for the transaction of business at any meeting.

VIII. There shall be three groups of Trustees:

(A) Thirty-two Trustees chosen by the Corporation, divided into four classes, each to serve four years; and in addition there shall be two groups of Trustees as follows:

(B) Trustees ex officio, who shall be the President and Vice President of the Corporation, the Director of the Laboratory, the Associate Director, the Treasurer, and the Clerk;

(C) Trustees Emeritus, who shall be elected from the Trustees by the Corporation. Any regular Trustee who has attained the age of seventy years shall continue to serve as Trustee until the next Annual Meeting of the Corporation, whereupon his office as regular Trustee shall become vacant and be filled by election by the Corporation and he shall become eligible for election as Trustee *Emeritus* for life. The Trustees *ex officio* and *Emeritus* shall have all the rights of the Trustees except that Trustees *Emeritus* shall not have the right to vote.

The Trustees and officers shall hold their respective offices until their successors are chosen and have qualified in their stead.

IX. The Trustees shall have the control and management of the affairs of the Corporation; they shall elect a President of the Corporation who shall also be Chairman of the Board of Trustees; and shall also elect a Vice President of the Corporation who shall also be the Vice Chairman of the Board of Trustees; they shall appoint a Director of the Laboratory; and they may choose such other officers and agents as they may think best; they may fix the compensation and define the duties of all the officers and agents; and may remove them, or any of them, except those chosen by the members, at any time; they may fill vacancies occurring in any manner in their own number or in any of the offices. The Board of Trustees shall have the power to choose an Executive Committee from their own number, and to delegate to such Committee such of their own powers as they may deem expedient. They shall from time to time elect members to the Corporation upon such terms and conditions as they may think best.

X. Any person interested in the Laboratory may be elected by the Trustees to a group to be known as Associates of the Marine Biological Laboratory.

XI. The consent of every Trustee shall be necessary to dissolution of the Marine Biological Laboratory. In case of dissolution, the property shall be disposed of in such manner and upon such terms as shall be determined by the affirmative vote of two-thirds of the Board of Trustees.

XII. The account of the Treasurer shall be audited annually by a certified public accountant.

XIII. These By-laws may be altered at any meeting of the Trustees, provided that the notice of such meeting shall state that an alteration of the By-laws will be acted upon.

IV. THE REPORT OF THE TREASURER

To the Trustees of the Marine Biological Laboratory:

Gentlemen:

Herewith is my report as Treasurer of the Marine Biological Laboratory for the year 1945.

The accounts have been audited by Messrs. Seamans, Stetson, and Tuttle, certified public accountants. A copy of their report is on file at the Laboratory and inspection of it by members of the Corporation will be welcomed.

The principal summaries of their report—The Balance Sheet, Statement of Income and Expense, and Current Surplus Account—are appended hereto as Exhibits A, B, and C.

The following are some general statements and observations based on the detailed reports:

I. Assets

1. Endowment Assets

As of December 31, 1945, the total book value of all the Endowment Assets, including the Scholarship Funds, was $966,772.16, a loss for the year of $17,128.41. The decline was due, as in the last two years, to losses on the mortgage participations on New York City realty held in the Trust Funds.

At the end of the year $831,993.01 was invested in marketable securities (bonds, preferred stocks and common stocks) with a market value of $910,162.31. $125,753.85 was invested in mortgage participations on New York City real estate. $9,025.30 was in uninvested principal cash.

The Treasurer's estimate of the actual value of the $125,753.85 in mortgage notes and participations held on December 31 is $85,750.00. With the market value of $910,162.31 on marketable securities and the $9,025.30 in cash this makes a total current valuation of $1,004,937.61 compared with total book value of $966,772.16.

The increase for the year in market values, $75,454.79, is largely due to the rise in common stock prices.

2. Plant Assets

There were no changes of any consequence in Plant Assets during the year. The Reserve Fund was increased nearly $10,000.00 to a total of $26,830.71 by the transfer of the Crane Co. dividends, part of the General Biological Supply House dividends and other items of current income.

3. Current Assets

The total of current assets increased $10,730.68 during 1945 to a total of $212,970.35. Current Liabilities at the end of the year were $2,754.70. Current Surplus increased $12,277.94 to a total of $196,337.90.

II. Income and Expenditures

The return to more normal operations for the Laboratory last summer resulted in larger totals for both income and expense items. Total income was $182,818.23, total expense including depreciation reserves of $26,968.12 was $173,044.95, giving a net surplus for the year of $9,773.28.

EXHIBIT A

Marine Biological Laboratory Balace Sheet, December 31, 1945

Assets

Endowment Assets and Equities:		
Securities and Cash in Hands of Central Hanover Bank and Trust Company, New York, Trustee	$ 950,130.04	
Securities and Cash in Minor Funds	16,642.12	
		$ 966,772.16

Plant Assets:			
Land	$ 111,425.38		
Buildings	1,326,345.54		
Equipment	187,837.87		
Library	337,266.01		
	$1,962,874.80		
Less Reserve for Depreciation	677,140.22	$1,285,734.58	
Reserve Fund, Securities and Cash		26,830.71	
Book Fund, Securities and Cash		18,282.46	
			$1,330,847.75
Current Assets:			
Cash		$ 30,467.02	
Accounts Receivable		20,396.05	
Inventories:			
Supply Department	$ 44,441.66		
Biological Bulletin	20,117.40	64,559.06	
Investments:			
Devil's Lane Property	$ 46,556.99		
Gansett Property	1,749.92		
Stock in General Biological Supply House, Inc.	12,700.00		
Other Investment Stocks	20,095.00		
Retirement Fund	11,517.82	92,619.73	
Prepaid Insurance		4,033.08	
Items in Suspense		895.41	
			$ 212,970.35
Total Assets			$2,510,590.26

MARINE BIOLOGICAL LABORATORY
WOODS HOLE
LIBRARY
MASS.

Liabilities

Endowment Funds:			
Endowment Funds	$ 948,646.82		
Reserve for Amortization of Bond Premiums	1,483.22		
		$ 950,130.04	
Minor Funds		16,642.12	
			$ 966,772.16
Plant Funds:			
Mortgage Notes Payable		$ 5,000.00	
Donations and Gifts	$1,172,564.04		
Other Investments in Plant from Gifts and Current Funds	153,283.71		
		$1,325,847.75	
			$1,330,847.75
Current Liabilities and Surplus:			
Accounts Payable		$ 2,754.70	
Items in Suspense		1,799.63	
Reserve for Repairs and Replacements		12,078.12	
Current Surplus		196,337.90	
			$ 212,970.35
Total Liabilities			$2,510,590.26

EXHIBIT B

Marine Biological Laboratory Income and Expense, Year Ended December 31, 1945

	Total		Net	
	Expense	Income	Expense	Income
Income:				
General Endowment Fund		$ 32,214.07		$ 32,214.07
Library Fund		9,479.18		9,479.18
Donations		755.00		755.00
Instruction	$ 9,554.39	7,220.00	$ 2,334.39	
Research	4,550.59	17,434.24		12,883.65
Evening Lectures	86.35		86.35	
Biological Bulletin and Membership Dues	6,393.65	8,775.63		2,381.98
Supply Department	39,255.03	47,812.56		8,557.53
Mess	24,146.52	20,750.36	3,396.16	
Dormitories	27,443.23	14,547.91	12,895.32	
(Interest and Depreciation charged to above 3 Departments)	(25,574.03)			25,574.03
Dividends, General Biological Supply House, Inc.		14,732.00		14,732.00
Dividends, Other Investment Stocks		725.00		725.00
Rents:				
Bar Neck Property	767.65	6,000.00		5,232.35
Janitor House	30.89	360.00		329.11
Danchakoff Cottages	240.86	275.00		34.14
Sale of Library Duplicates, Micro Film, etc.		344.74		344.74
Microscope and Apparatus Rental		1,372.54		1,372.54
Sundry Income		20.00		20.00
Maintenance of Plant:				
Buildings and Grounds	23,642.27		23,642.27	
Apparatus Department	4,911.52		4,911.52	
Chemical Department	2,265.30		2,265.30	
Library Expense	6,487.95		6,487.95	
Workmen's Compensation Insurance	526.63		526.63	
Truck Expense	238.60		238.60	
Bay Shore Property	92.78		92.78	
Great Cedar Swamp	21.00		21.00	
General Expenses:				
Administration Expense	15,168.99		15,168.99	
Endowment Fund Trustee and Safe-Keeping	1,028.45		1,028.45	
Bad Debts	375.97		375.97	
Special Repairs on account of 1944 Hurricane Damage	4,297.24		4,297.24	
Interest	125.00		125.00	
Reserve for Depreciation	26,968.12		26,968.12	
	$173,044.95	$182,818.23	$104,862.04	$114,635.32
Excess of Income over Expense carried to Current Surplus	9,773.28		9,773.28	
	$182,818.23		$114,635.32	

EXHIBIT C

Marine Biological Laboratory, Current Surplus Account, Year Ended December 31, 1945

Balance January 1, 1945			$184,059.96
Add:			
Excess of Income over Expense for Year as shown in Exhibit B		$ 9,773.28	
Gain on Gansett Lots Sold		464.18	
Bad Debts Recovered		82.23	
Mortgage Payable, Transferred to Plant Funds		5,000.00	
Reserve for Depreciation Charged to Plant Funds		26,968.12	42,287.81
			$226,347.77
Deduct:			
Payments from Current Funds during Year for Plant Assets as shown in Schedule IV:			
Buildings	$ 7,402.65		
Equipment	4,462.50		
Library	7,500.43		
	$19,365.58		
Less Received for Plant Assets Sold	5,600.00		
		$13,765.58	
Pensions Paid	$ 3,460.00		
Loss on Retirement Fund Securities	847.32		
	$ 4,307.32		
Less Retirement Fund Income	311.79		
		$ 3,995.53	
Transfers to Reserve Fund:			
Portion of Dividends from General Biological Supply House, Inc.	$ 2,500.00		
Dividends from Crane Co.	625.00		
Income from Operation and Sale of Property 445–51 W. 23rd and 450–2 W. 24th Sts., N. Y. C.	8,947.72		
Gansett Property Profits, 1944	176.04	12,248.76	30,009.87
Balance, December 31, 1945			$196,337.90

MARINE BIOLOGICAL LABORATORY LIBRARY WOODS HOLE, MASS.

Respectfully submitted,
Donald M. Brodie,
Treasurer

V. REPORT OF THE LIBRARIAN

The sum $12,262.54 appropriated to the library in 1945 was expended as follows: books, $469.99; serials, $2,625.79; binding, $577.60; express, $43.22; supplies, $147.84; salaries, $7,262.54 ($1,150 of this sum was contributed by the Woods Hole Oceanographic Institution); back sets, $1,104.98; insurance, $45.00;

sundries, $5.00; total, $12,281.96. The cash receipts of the library totalled $344.74: for microfilms, $220.34 ($62.17 expenses paid by the library and accounted above under "supplies"); sale of duplicates, $122.74; sale of the "Serial List," Biological Bulletin supplement number, $1.66. This sum, $344.74, reverts to the laboratory and does not include rent payments for library readers which are collected by the main office. There were 49 library readers accommodated in the library during the summer of 1945.

Of the Carnegie of New York Fund, $126.35 was expended for the completion of one journal and the partial completion of another.

The sum appropriated by the Woods Hole Oceanographic Institution in 1945 for purchases was $800. A balance of $949.39 remaining from 1944 made an available total of $1,749.39. Of this sum $947.12 was expended, leaving a balance of $802.27 towards future purchases. In addition to the above, the Woods Hole Oceanographic Institution contributed $1,150 (see above under salaries).

During 1945 the library received 902 current journals: 279 (8 new) by subscription to the Marine Biological Laboratory; 30 (7 new) to the Woods Hole Oceanographic Institution; exchanges 352 (6 new; 145 reinstated foreign) and 58 (35 foreign reinstated) with the Woods Hole Oceanographic Institution publications; 177 as gifts to the former and 6 to the latter. The library acquired 206 books: 77 by purchase of the Marine Biological Laboratory; 44 by purchase of the Woods Hole Oceanographic Institution; 10 gifts by the authors; 46 gifts by the publishers; 20 by miscellaneous donors and 9 from Miss Jane Strong. There were 22 back sets of serial publications completed: 14 purchased by the Marine Biological Laboratory (one with the Carnegie Fund); 2 by the Woods Hole Oceanographic Institution; 5 by exchange of the "Biological Bulletin" and one by exchange of duplicate material. Partially completed sets were 54: purchased by the Marine Biology Laboratory, 27 (1 by the Carnegie Fund); purchased by the Woods Hole Oceanographic Institution, 2; by exchange with the "Biological Bulletin," 2; by gift and exchange of duplicate material, 23.

The reprint additions to the library were 4,620; current of 1944, 604; current of 1945, 64; and of previous dates, 3,952. A total of 6,390, 2,130 not duplicates of our holdings, were presented to the library; 4,295 by Mrs. Meigs; 67 by Dr. H. G. Cassidy; 627 by the University of Utah; 26 by Dr. B. M. Davis; and 1,375 by Dr. L. C. Wyman. The large collection of Dr. Garrey's reprints presented last year have not as yet been counted nor started on the way toward cataloguing.

At the end of the year 1945 the library contained 53,990 bound volumes and 137,674 reprints.

Readers of the library report will be glad to note the number of foreign exchanges that have already been reinstated during 1945, both for the "Biological Bulletin" and for the Woods Hole Oceanographic Institution publications: 145 for the former and 35 for the latter. Next year's report will probably show the pre-war number reinstated save only for Germany and perhaps for Russia since we get very poor response from that country. Nor have we heard anything in regard to the German journals on order with Otto Harrassowitz which are apparently stalled if not destroyed in Leipzig. The library committee that is working with the State Department to get these released has nothing so far to report to this library.

VI. REPORT OF THE DIRECTOR

To the Trustees of The Marine Biological Laboratory:

Gentlemen:

I herewith submit a report on the fifty-eighth session of the Marine Biological Laboratory.

1. Attendance

Since 1943 our total attendance has increased from the low point reached that year. In 1944 it was 53 per cent of the pre-war average of 490; in 1945 it was 63 per cent. This increase is found among the independent investigators and the students; the beginning investigators and research assistants, who, as I explained in the last report, belong in one group, are still sparsely represented. In 1945 there were only 36, whereas the pre-war average was 130. The advance registration for 1946 shows an encouraging increase in this group. Many of the applicants are veterans who are taking advantage of Government funds provided under the G. I. Bill of Rights.

2. Building Repairs

One of the inevitable effects of the war has been the deterioration of our buildings. Lack of materials and labor has up until now prevented all but the most essential repairs from being made. Fortunately, we are now able to begin to put our house in order, in spite of the shortage of some critical materials. To determine what work should be done, a Committee on Special Repairs, under the able leadership of Mr. C. L. Claff, conducted a thorough survey of all of the buildings and made detailed recommendations. The report, a model of completeness as drawn up by Mr. Claff, calls for the ultimate expenditure of approximately $145,000 for present repairs and future desirable improvements not only in the buildings but also in equipment for the Apparatus Department and the Supply Department.

The Executive Committee voted to expend the entire Reserve Fund, amounting to $25,000, and all but a minimum of the current cash on hand for making the most urgently needed repairs at once. It also laid plans for securing outside funds with which to complete the changes called for in the report, and to purchase apparatus. In addition, funds for a new building and for additional endowment are to be sought.

Many of the essential repairs have already been made. The Mess kitchen, never properly restored after the Navy occupation, is now in good condition, and improvements in the dining room have been made. The Botany Building, unused for several seasons, has been put to rights with new plumbing, wall tables, shelves, and other fixtures. Replacements have been made in the Supply Department and Rockefeller Building; hot water systems are installed in the residences heretofore not so provided; and much painting has been done. This work was accomplished in large measure by our permanent staff, under the direction of Mr. MacNaught. All the men worked faithfully and energetically, and have completed the assigned tasks in a most satisfactory manner. It is hoped that waterproofing of the Crane and Brick Buildings may be completed before the 1946 season begins. As soon as this has been satisfactorily finished, those Laboratory rooms which have been damaged by water can be made presentable.

3. The Housing Problem

An unexpected outcome of war-time activities is the housing shortage. Before the war our residences and the houses in the village could accommodate 450 to 500 persons during the course of a summer. But when the Oceanographic Institution embarked on extensive defense projects, the number of its workers increased from comparatively few to upwards of 250, most of whom are year round residents. They now occupy most of the available houses in the village; some are forced to live as far away as North Falmouth and Hyannis. As a result of this crowding we shall be unable, in the summer of 1946, to take care of more than 375 investigators and students—that is, about 100 less than our pre-war average. Indeed, we can accommodate this number only because the authorities of the U. S. Fish and Wild Life Commission have granted us the use of a part of the Fisheries residence. For their cooperation in this, and in many other ways, the Laboratory is grateful.

When it is possible once more to build houses, some of this pressure for living space will be relieved. To encourage investigators to have homes here in Woods Hole, the Laboratory has opened up the Devil's Lane tract, situated a mile and more from the center of the village, between the State Road to Falmouth and the railroad. About 100 lots will presently be available.

In the meantime, the number of applicants for research space will undoubtedly increase, and we shall be unable to find places for all qualified investigators who wish to come. The Administration thus faces the unwelcome prospect of having to choose between applicants. The Executive Committee has ruled that investigators, instructors, and students should have preference over Library readers in the residences and at the Mess. But some further method of selection must be followed until the housing shortage is relieved.

4. Financial Problems

The report of the Treasurer shows that our financial condition is sound; that is, we are free from debt, and have about $57,000 in the Reserve and Current Cash accounts. But most of this has already been ear-marked to pay for the most necessary repairs, and for foreign journals not yet delivered. We shall still need a larger amount for other needed repairs and replacements. When these have been made we can say that our regular income from all sources is sufficient to maintain the Laboratory on its present basis. But in order to expand our research facilities we must have additional funds. It is estimated that $30,000 each year should be spent for this purpose.

5. Gifts

Mr. Allen R. Memhard has provided a fund of $1,000, the income of which may be awarded to a qualified student who has completed the Embryology course.

Mrs. Adele K. Stricker has presented to the Laboratory the sum of $50 in memory of her son, Capt. George J. Stricker, who worked here during the summers of 1933 and 1934.

Dr. A. C. Redfield contributed $100 for a hedge and trees to be planted to the east of the Stone Building.

Donations for current purposes received during the year were as follows: Mrs. E. B. Meigs, $25.00; Dr. William D. Curtis, $100.00; M. B. L. Associates, $630.00.

6. Deaths

This year we have sustained irreparable losses by death; Dr. T. H. Morgan, Trustee since 1897, whose scientific achievements and devotion to this Laboratory from its earliest days contributed greatly to its growth in usefulness and influence, and Dr. C. E. McClung, elected Trustee in 1913, active in all Laboratory affairs, especially in the building up of our great Library.

7. Election of Trustees

At the meeting of the Corporation, held August 14, 1945, the following were elected Trustees Emeriti: Dr. F. P. Knowlton, elected Trustee in 1922; Dr. R. S. Lillie, elected Trustee in 1921.

The following were elected Trustees: Dr. P. B. Armstrong, Professor of Anatomy, College of Medicine, Syracuse University; Dr. A. K. Parpart, Associate Professor of Biology, Princeton University.

8. Publications

The Executive Committee voted to print in this Report a list of papers, based wholly or in part on work done at this Laboratory, and published during the years 1941–1945. A similar list, which appeared in the Annual Report of 1908, covered the years from the beginning of the Laboratory in 1888 to 1907. It is hoped that eventually a complete compilation of titles to include the intervening years may be made.

Appended as parts of this Report are:

1. Publications from this Laboratory during the years 1941–1945.
2. The Staff.
3. Investigators and Students.
4. Tabular View of Attendance, 1941–1945.
5. Subscribing and Cooperating Institutions.
6. Evening Lectures.
7. Shorter Scientific Papers.
8. Members of the Corporation.

Respectfully submitted,
CHARLES PACKARD,
Director

1. PUBLICATIONS FROM THE MARINE BIOLOGICAL LABORATORY, 1941–1945

Note: An asterisk before a title indicates that the work was done only in part at this Laboratory.

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WOODRUFF, L. L. *See* Boell and Woodruff, 1941.

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2. The Staff, 1945

Charles Packard, Director, Marine Biological Laboratory, Woods Hole, Massachusetts.

Senior Staff of Investigation

E. G. Conklin, Professor of Zoology, Emeritus, Princeton University.
Frank R. Lillie, Professor of Embryology, Emeritus, The University of Chicago.
Ralph S. Lillie, Professor of General Physiology, Emeritus, The University of Chicago.
C. E. McClung, Professor of Zoology, Emeritus, University of Pennsylvania.
S. O. Mast, Professor of Zoology, Emeritus, Johns Hopkins University.
A. P. Mathews, Professor of Biochemistry, Emeritus, University of Cincinnati.
T. H. Morgan, Director of the Biological Laboratory, California Institute of Technology.
G. H. Parker, Professor of Zoology, Emeritus, Harvard University.

ZOOLOGY

I. Consultants

T. H. Bissonnette, Professor of Biology, Trinity College.
L. L. Woodruff, Professor of Protozoology, Yale University.

II. Instructors

F. A. Brown, Associate Professor of Zoology, Northwestern University, in charge of course.
T. H. Bullock, Assistant Professor of Neurology University of Missouri Medical School.
W. D. Burbanck, Associate Professor of Biology, Drury College.
C. G. Goodchild, Associate Professor of Biology, Southwest Missouri State Teachers College.
John H. Lochhead, Instructor in Zoology, University of Vermont.
Madelene E. Pierce, Assistant Professor of Zoology, Vassar College.
W. M. Reid, Assistant Professor of Biology, Monmouth College.
Mary D. Rogick, Professor of Biology, College of New Rochelle.

III. Laboratory Assistant

Antoin Baca, Duke University Medical School.

EMBRYOLOGY

I. Consultants

H. B. Goodrich, Professor of Biology, Wesleyan University.

II. INSTRUCTORS

W. W. BALLARD, Professor of Zoology, Dartmouth College.
DONALD P. COSTELLO, Professor of Zoology, University of North Carolina.
VIKTOR HAMBURGER, Professor of Zoology, Washington University, in charge of course.
JANE M. OPPENHEIMER, Assistant Professor in Biology, Bryn Mawr College.

III. RESEARCH ASSISTANT

MARJORIE HOPKINS, University of California.

IV. LABORATORY ASSISTANTS

CATHERINE HENLEY, The Johns Hopkins University.
ELEANOR LERNER, Washington University.

PHYSIOLOGY

I. CONSULTANTS

WILLIAM R. AMBERSON, Professor of Physiology, University of Maryland, School of Medicine.
HAROLD C. BRADLEY, Professor of Physiological Chemistry, University of Wisconsin.
WALTER E. GARREY, Professor of Physiology, Vanderbilt University Medical School.
MERKEL H. JACOBS, Professor of Physiology, University of Pennsylvania.

II. INSTRUCTORS

ROBERT BALLENTINE, Lecturer in Zoology, Columbia University (absent in 1945).
AURIN CHASE, Assistant Professor of Biology, Princeton University.
ARTHUR C. GIESE, Associate Professor of Biology, Stanford University (absent in 1945).
E. S. GUZMAN BARRON, Associate Professor of Biochemistry, The University of Chicago.
RUDOLF T. KEMPTON, Professor of Zoology, Vassar College (absent in 1945).
ARTHUR K. PARPART, Associate Professor of Biology, Princeton University, in charge of course.
ROBERT RAMSEY, Associate Professor of Physiology, Medical College of Virginia.

BOTANY

I. CONSULTANTS

S. C. BROOKS, Professor of Zoology, University of California.
B. M. DUGGAR, Professor of Plant Physiology, University of Wisconsin.

II. INSTRUCTORS

WM. RANDOLPH TAYLOR, Professor of Botany, University of Michigan, in charge of course.
HANNAH CROASDALE, Technical Assistant, Dartmouth College.

EXPERIMENTAL RADIOLOGY

G. FAILLA, Memorial Hospital, New York City.
L. ROBINSON HYDE, Phillips Exeter Academy, Exeter, N. H.

LIBRARY

PRISCILLA B. MONTGOMERY (MRS. THOMAS H. MONTGOMERY, JR.), Librarian
DEBORAH LAWRENCE MRS. ELON H. JESSUP MARY A. ROHAN

APPARATUS DEPARTMENT

E. P. Little, Phillips Exeter Academy, Exeter, N. H., Manager
J. D. Graham
Dorothy LeFevre

CHEMICAL DEPARTMENT

E. P. Little, Phillips Exeter Academy, Exeter, N. H., Manager

SUPPLY DEPARTMENT

James McInnis, Manager
D. J. Zinn, Naturalist
Ruth Crowell
Grace M. Kennerson
M. B. Gray
W. E. Kahler
F. N. Whitman
A. M. Hilton
G. Lehy

GENERAL OFFICE

F. M. MacNaught, Business Manager
Polly L. Crowell
Mrs. Lila S. Myers

GENERAL MAINTENANCE

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W. C. Hemenway
G. T. Nickelson, Jr.
R. W. Kahler
T. E. Tawell
A. J. Pierce

THE GEORGE M. GRAY MUSEUM

George M. Gray, Curator Emeritus

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3. Investigators and Students

Independent Investigators, 1945

Abell, Richard G., Assistant Professor of Anatomy, University of Pennsylvania.
Addison, William H. F., Professor of Normal Histology and Embryology, University of Pennsylvania.
Anderson, Rubert S., Assistant Professor of Physiology, University of Maryland.
Anfinson, Christian B., Jr., Instructor in Biological Chemistry, Harvard Medical School.
Armstrong, Philip B., Professor of Anatomy, Syracuse University.
Aronoff, Samuel, Instructor, University of Chicago.
Axelrad, Arthur A., Investigator, McGill University.
Ball, Eric G., Associate Professor of Biological Chemistry, Harvard Medical School.
Ballard, W. W., Professor of Zoology, Dartmouth College.
Ballentine, Robert, Instructor, Columbia University.
Barron, E. S. Guzman, Associate Professor of Biochemistry, The University of Chicago.
Barth, L. G., Associate Professor of Zoology, Columbia University.
Beers, Charles Dale, Professor of Zoology, University of North Carolina.
Berger, Charles A., Professor of Cytology, Fordham University.
Bertholf, Lloyd M., Professor of Biology, Western Maryland College.
Bevelander, Gerrit, Associate Professor of Anatomy, New York University.
Bliss, Alfred F., Instructor in Physiology and Pharmacology, Union University.
Bodian, David, Associate in Epidemiology, Johns Hopkins University.

Bronk, Detlev W., Professor of Biophysics, Johnson Foundation.
Brooks, Matilda M., Research Associate in Biology, University of California.
Brooks, Sumner C., Professor of Zoology, University of California.
Brown, Dugald E. S., Professor of Physiology, New York University.
Brown, Frank A., Jr., Associate Professor of Zoology, Northwestern University.
Brownell, Katharine A., Research Associate, Ohio State University.
Budington, Robert A., Professor of Zoology, Emeritus, Oberlin College.
Bullock, Theodore H., Assistant Professor of Anatomy, University of Missouri.
Burbanck, William D., Associate Professor of Biology, Drury College.
Burkholder, Paul R., Professor of Botany, Yale University.
Chambers, Robert, Research Professor of Biology, New York University.
Chase, Aurin M., Assistant Professor of Biology, Princeton University.
Cheney, Ralph H., Chairman Biology Department, Long Island University.
Chidester, F. E., Research Worker, Lee Foundation.
Claff, C. Lloyd, Research Fellow in Surgery, Harvard Medical School.
Clark, Eleanor L., Voluntary Research Worker, University of Pennsylvania.
Clark, Eliot R., Professor of Anatomy, University of Pennsylvania.
Claude, Albert, The Rockefeller Institute for Medical Research.
Clement, A. C., Associate Professor in Biology, College of Charleston.
Clowes, G. H. A., Director of Research, Lilly Research Laboratories.
Conklin, Edwin G., Professor of Zoology, Emeritus, Princeton University.
Copeland, Manton, Professor of Biology, Bowdoin College.
Costello, Donald P., Professor of Zoology, University of North Carolina.
Crampton, Henry E., Professor Emeritus, Columbia University.
Croasdale, Hannah T., Technical Assistant, Dartmouth College.
Crouse, Helen V., Research Associate, University of Pennsylvania.
Crowell, Sears, Assistant Professor of Zoology, Miami University.
Froehlich, Alfred, Associate, May Institute for Medical Research.
Furchgott, Robert F., Research Associate, Cornell University.
Furth, Jacob, Professor of Pathology, Cornell University.
Gaffron, Hans, Assistant Professor of Biochemistry, Research Associate, University of Chicago.
Galtsoff, Paul S., Senior Biologist, U. S. Fish and Wildlife Service.
Garrey, W. E., Professor of Physiology, Emeritus, Vanderbilt University, School of Medicine.
Glaser, Otto C., Professor of Biology, Amherst College.
Goodchild, Dr. C. G., Associate Professor of Biology, State Teachers College.
Gorbman, Aubrey, Instructor in Biology, Wayne University.
Gould, Harley N., Professor of Biology, H. Sophie Newcomb College.
Grand, C. G., Research Associate, New York University.
Hamburger, Viktor, Professor of Zoology, Washington University.
Hartman, Frank A., Professor and Chairman of Department of Physiology, Ohio State University.
Harvey, Ethel Browne, Independent Investigator Biology Department, Princeton University.
Harvey, E. Newton, Professor of Physiology, Princeton University.
Hayashi, Teru, Instructor in Zoology, University of Missouri.
Haywood, Charlotte, Professor of Physiology, Mount Holyoke College.
Heilbrunn, L. V., Professor of Zoology, University of Pennsylvania.
Hibbard, Hope, Professor of Zoology, Oberlin College.
Hickson, Anna Keltch, Research Chemist, Eli Lilly & Company.
Hopkins, Hoyt S., Associate Professor of Physiology, New York University, College of Dentistry.
Huber, Wolfgang, Senior Research Chemist, Winthrop Chemical Company.
Jacobs, M. H., Professor of General Physiology, University of Pennsylvania.
Jaeger, Lucena, Research Associate, Columbia University.
Jenkins, George B., Professor of Anatomy, Emeritus, George Washington University.
Johlin, J. M., Associate Professor of Biochemistry, Vanderbilt University.
Johnson, Frank H., Assistant Professor of Biology, Princeton University.
Krahl, M. E., Instructor in Pharmacology, Columbia University.
Landis, Eugene M., Professor of Physiology and Head of Department, Harvard Medical School.

Lavin, George I., In charge of Spectroscopic Laboratory, Rockefeller Institute for Medical Research.

Lee, Richard E., Student, Columbia University.

Lieben, Fritz, Research Fellow, Johns Hopkins University.

Lillie, Ralph S., Professor of Physiology, Emeritus, University of Chicago.

Lochhead, John H., Assistant Professor of Zoology, University of Vermont.

McClung, C. E., Professor of Zoology, Emeritus, University of Pennsylvania.

MacLean, Bernice L., Assistant Professor, Department Biological Sciences, Hunter College.

Magalhaes, Hulda, Instructor in Zoology, Duke University.

Marks, Mildred H., Student, Massachusetts Institute of Technology.

Marsland, Douglas A., Associate Professor of Biology, New York University.

Mast, S. O., Professor of Zoology, Emeritus, Johns Hopkins University.

Mathews, Albert P., Professor of Biochemistry, Emeritus, University of Cincinnati.

Matthews, Samuel A., Professor of Biology, Williams College.

Memhard, Allen R., Crescent Road, Riverside, Connecticut.

Menkin, Valy, Assistant Professor of Pathology, Duke University.

Metz, Charles W., Director Zoological Laboratory, University of Pennsylvania.

Michaelis, Leonor, Member Emeritus, Rockefeller Institute for Medical Research.

Nachmansohn, David, Research Associate in Neurology, Columbia University.

Northrop, John H., Member of the Institute, Rockefeller Institute for Medical Research.

Oppenheimer, Jane M., Assistant Professor of Biology, Bryn Mawr College.

Osterhout, W. J. V., Member Emeritus, Rockefeller Institute for Medical Research.

Parpart, Arthur K., Associate Professor of Biology, Princeton University.

Pierce, Madelene E., Associate Professor of Zoology, Vassar College.

Ramsey, Robert W., Associate Professor of Physiology, Medical College of Virginia.

Rankin, John S., Jr., Assistant Professor of Zoology, University of Connecticut.

Reid, W. Malcolm, Assistant Professor of Biology, Monmouth College.

Riker, Walter F., Jr., Instructor in Medicine and Pharmacology, Cornell University Medical School.

Ris, Hans, Assistant in Physiology, Rockefeller Institute for Medical Research.

Robbie, Wilbur A., Research Associate, State University of Iowa.

Rogick, Mary Dora, Professor of Biology, College of New Rochelle.

Sampson, Myra M., Professor of Zoology, Smith College.

Sandow, Alexander, Assistant Professor of Biology, New York University.

Schaeffer, A. A., Professor of Biology, Temple University.

Scharrer, Ernst A., Assistant Professor of Anatomy, Western Reserve University School of Medicine.

Scott, Sister Florence Marie, Professor of Zoology, Seton Hill College.

Scott, George T., Instructor, Oberlin College.

Shanes, Abraham M., Assistant Professor of Physiology, New York University College of Dentistry.

Shapiro, Harry H., Assistant Professor of Anatomy, Columbia University.

Slifer, Eleanor H., Assistant Professor of Zoology, State University of Iowa.

Smith, Dietrich Conrad, Associate Professor of Physiology, University of Maryland, School of Medicine.

Stern, Kurt G., Lecturer in Department of Chemistry, Polytechnic Institute of Brooklyn.

Stewart, Dorothy R., Fellow in Physiology, University of Pennsylvania.

Stokey, Alma G., Professor of Plant Science, Emeritus, Mount Holyoke College.

Stunkard, H. W., Professor of Biology, New York University.

Taft, Charles H., Associate Professor of Pharmacology, Medical Branch, University of Texas.

Taylor, William Randolph, Professor of Botany, University of Michigan.

TeWinkel, Lois E., Associate Professor of Zoology, Smith College.

Thivy, Francesca, Professor of Biology, Women's Christian College.

Villee, Claude A., Assistant Professor of Zoology, University of North Carolina.

Wainio, Walter W., Assistant Professor of Physiology, New York University, College of Dentistry.

Warren, Charles O., Assistant Professor of Physiology, Cornell University Medical College.

Wenrich, D. H., Professor of Zoology, University of Pennsylvania.

Whiting, Anna R., Visiting Investigator, University of Pennsylvania.

Whiting, P. W., Associate Professor of Zoology, University of Pennsylvania.
Wichterman, Ralph, Assistant Professor of Biology, Temple University.
Willier, B. H., Professor of Zoology and Director of the Biological Laboratories, Johns Hopkins University.
Winsor, Charles P., Research Associate, Princeton University.
Witkus, Eleanor R., Instructor in Botany and Bacteriology, Fordham University.
Woodward, Alvalyn E., Assistant Professor, University of Michigan.
Woodward, Arthur A., Jr., Research Assistant, University of Pennsylvania.
Wooley, D. W., Associate, Rockefeller Institute for Medical Research.
Wrinch, Dorothy, Lecturer in Physics, Smith College.
Yntema, Chester L., Assistant Professor of Anatomy, Cornell University Medical College.
Zweifach, Benjamin W., Research Associate in Biology, New York University.

Beginning Investigators, 1945

Brown, Ellen, Commonwealth Fellow, University of California Medical School.
Brown, Virginia H., Graduate Student, Ohio State University.
Coyne, Christopher J., Student, University of Pennsylvania.
Davidson, Margaret E., Demonstrator and Assistant to Dr. Berrill, McGill University.
Krugelis, Edith J., Graduate Student, Columbia University.
Lerner, Eleanor, Fellow in Zoology, Washington University.
Lovelace, Roberta, Teaching Fellow, University of North Carolina.
Miller, Helma C., Assistant, Johns Hopkins University.
Schneyer, Leon H., Instructor, New York University, College of Dentistry.
Wilson, Walter, Graduate Student, University of Pennsylvania.

Research Assistants, 1945

Abramsky, Tess, Research Assistant, Rockefeller Institute for Medical Research.
Brunelli, Eleanor L., Research Assistant, New York University, College of Dentistry.
DeFalco, Rose H., Research Assistant, University of Pennsylvania.
Fischl, Mathilda, Research Assistant in Medicine, Cornell University.
Franz, Ruth Estelle, Research Assistant, Yale University.
Garzoli, Ray F., Graduate Student, University of California.
Gould, David, Research Technician, New York University.
Harlow, Janet, Technician, Syracuse University.
Helfman, Myrna, Technician, New York University.
Henley, Catherine, Graduate Teaching Assistant, Johns Hopkins University.
Honegger, Carol, Student, Temple University.
Lawler, H. Claire, Research Assistant, New York University.
Levin, Isaac, Research Assistant, Princeton University.
Levy, Betty, Research Assistant, Rockefeller Institute for Medical Research.
Loofbourrow, G. N., Instructor, Rhode Island State College.
McVeigh, Ida, Research Assistant in Botany, Yale University.
Metz, Delilah B., Research Assistant, Eli Lilly & Co.
Miner, Karyl, Research Assistant, New York University.
Mitchell, Constance, Research Assistant, University of Pennsylvania.
Pettengill, Olive S., Student, Temple University.
Quinn, Gertrude P., Research Assistant, New York University.
Rothenberg, M. A., Research Assistant in Biochemistry, College of Physicians and Surgeons.
Uhlman, Gloria E., Research Assistant, Yale University.
Walters, C. Patricia, Research Assistant, Eli Lilly & Co.
Warner, Charlotte, Medical Student, University of Pennsylvania.
Zacks, Sumner I., Student, Brookline High School.

Library Readers, 1945

Amberson, William R., Professor of Physiology, University of Maryland.
Beck, Lyle V., Associate Professor of Physiology, Hahnemann Medical College.

Bendich, Aaron, Member War Research Division, Neurological Institute, New York.
Block, Richard J., Associate, New York Medical College.
Brehme, Katherine S., Lecturer, Cornell University Medical College.
Cahen, Raymond L., Research Assistant, Yale University, Medical School.
Carson, Hampton L., Instructor in Zoology, Washington University.
Cassidy, Harold G., Assistant Professor of Chemistry, Yale University.
Colwin, Laura Hunter, Instructor, Pennsylvania College for Women.
Friedemann, Ulrich H., Head of Department of Bacteriology, Brooklyn Jewish Hospital.
Frisch, John A., Professor of Biology, Canisius College.
Gates, R. Ruggles, Professor Emeritus, University of London.
Gurewich, Vladimir, Assistant Visiting Physician, Bellevue Hospital.
Kabat, Elvin A., Research Associate in Biochemistry, College of Physicians and Surgeons.
Kaylor, Cornelius T., Assistant Professor of Anatomy, Syracuse University.
Keller, Rudolph, Researcher, Robinson Foundation, New York.
Krasnow, Frances, Head of Department of Research, Guggenheim Dental Foundation.
Lange, Mathilde M., Professor of Zoology, Head of Department of Biology, Wheaton College.
Loewi, Otto, Research Professor of Pharmacology, New York University, College of Medicine.
Marinelli, Leonidas, Physicist, Memorial Hospital.
Mavor, James W., Professor of Biology, Union College.
Mayer, Manfred M., Scientific Staff, War Research Division, Columbia University.
Metz, Charles B., Instructor in Biology, Wesleyan University.
Meyerhof, Dr. Otto, Research Professor of Biochemistry, University of Pennsylvania.
Moldaver, Joseph, Research Associate in Neurology, Columbia University.
Moore, John A., Assistant Professor of Zoology, Barnard College.
Moschcowitz, Eli, Assistant Professor of Chemical Medicine, Columbia University.
Oseasohn, Robert O., Long Island College of Medicine.
Perry, Barbara H., Graduate Student and Teaching Fellow in Zoology, Smith College.
Ponder, Eric, Research, Nassau Hospital.
Ramsden, Ethel J., Instructor in Biology, Montclair Teachers College.
Robinson, Miles H., Instructor in Pharmacology, University of Pennsylvania.
Ryan, Francis J., Assistant Professor, Columbia University.
Scott, Allan, Assistant Professor of Biology, Union College.
Strauss, William L., Jr., Associate Professor of Anatomy, Johns Hopkins University.
VonDach, Herman, Assistant Professor of Physiology, Georgetown Medical School.
Wallach, Jacques B., Long Island College of Medicine.
Zorzoli, Anita, Assistant Instructor, New York University.

Students, 1945

BOTANY

Barraclough, Mary Edith, Student, Smith College.
Dietz, Alma, Assistant in Biology, American International College.
Gardner, Elizabeth B., Radcliffe College.
Moul, Edwin Theodore, Botany Assistant, University of Pennsylvania.
Smith, Mattie Lou, Student, Radcliffe College.

EMBRYOLOGY

Beach, Janet, Student, University of Connecticut.
Bernier, Germaine, University of Montreal, Quebec, Canada.
Berry, Beth Sinclair, Student, Rockford College, Illinois.
Carter, Marjorie Estelle, Teacher, Georgia State Women's College.
Chirico, Anna Marie, Student, Seton Hill College.
Clark, Carl Cyrus, Student, Amherst College.
Copinger, Anne Stevens, Goucher College.
Ehrlich, Miriam, Knox College.
Izzo, Mary Jane, University of Rochester.
Kell, Amy, University of Illinois.
Levin, Ilane B., Goucher College.

Lodico, Dorothy Geraldine, University of Rochester.
Lovelace, Lollie Roberta, Teaching Fellow, University of North Carolina.
Marker, Muriel Josephine, Student, Colby College.
Mezger, Liselotte, Student, Bryn Mawr College.
Miller, Helma C., Graduate Assistant, Johns Hopkins University.
Perkins, Barbara Burnham, University of Connecticut.
Raymond, Barbara, Student, Swarthmore College.
Rice, Mary Esther, Assistant in Biology Laboratory, Drew University.
Roberts, Elizabeth S., Assistant in Biology, Wilson College.
Ruderman, Claire, Teaching Assistant, University of Rochester.
Thorby, Jean Adelaide, Student, Rockford College.
Uphoff, Delta E., University of Rochester.

PHYSIOLOGY

Brust, Manfred, Student, New York University.
Cook, John Alfred, George Washington University.
Ferguson, Alice Howard, Graduate Assistant, Louisiana State University.
Flinker, Marie-Louise M., Assistant in Physiology, Vassar College.
Fogerson, Virginia Lee, Student, Drury College.
Foster, Elizabeth Jane, Student, University of Illinois.
Goldsmith, Yvette, Perth Amboy, New Jersey.
Hajek, Norma Mary, Cornell University.
Hecht, Liselotte Isabella, Student, University of Michigan.
Resnick, Oscar, Resident Scholar, Harvard University.
Weiss, Michael S., Student, Washington Square College.
Wolff, Mary Lyda, Instructor, Cedar Crest College.
Worken, Barney, 3400 Wayne Avenue, New York City.

ZOOLOGY

Aronowitz, Olga, New York University.
Bates, Mary Florence, Student, Vassar College.
Bayors, Winifred M., Student Seton Hill College.
Beal, Judith D., Vassar College.
Benjamin, Mrs. Rezsin C., Undergraduate Student, University of Rochester.
Bernard, Sister Marie, Fordham University.
Bernier, Germaine, Instructor, University of Montreal.
Bezilla, Helen, Student, Seton Hill College.
Bradin, John L., Northwestern University.
Calvert, Julie Neil, Student, Wilson College.
Carlson, Alice Marie, Laboratory Assistant, University of Minnesota.
Chaffin, Evelyn L., Student, Drury College.
Clark, Carl Cyrus, Amherst College.
Cummings, Rev. George W., Graduate Student, Catholic University.
Dailey, Dorothy Helen, Depauw University.
Dawson, Mary Jean, Student, Mt. Holyoke College.
Dempsey, Ellen, Oberlin College.
Dickason, Mary Elizabeth, Student, Smith College.
Farnham, Carol Jean, Student, Drury College.
Freitag, Janet Faith, Student, University of Connecticut.
Goldis, Bernice Ruth, Graduate Student, University of Pennsylvania.
Hanlon, Rev. James J., Graduate Student, Fordham University.
Hill, Shirley B., Student, Vassar College.
Hines, Eileen Barbara, State University of Iowa.
Jones, Dorothy B., Student, University of Connecticut.
Josita, Sister M., Student, Fordham University.
Julier, Edith Vaillant, Student, Vassar College.

Krekeler, Carl H., Student, Washington University.
Kuhn, Alice Roberts, Western-Maryland College.
Lowens, Mary Dorothy, Student, Swarthmore College.
McClain, Marylow, Student, Swarthmore College.
McGregor, Elizabeth, Instructor, Mount Holyoke College.
McVicker, Sister Maureen, Teacher of Biology, St. Joseph's College for Women.
Malloch, Jean, Vassar College.
Meihack, Helen Lloyd, Student, Oberlin College.
Mina, Frank A., Laboratory Instructor, Fordham University.
Osborn, Joan A., Student, Barnard College.
Peters, Rev. Joseph J., Graduate Student, Fordham University.
Raymond, Barbara, Student, Swarthmore College.
Riggs, Austin F., Student, Harvard University.
Rogers, Henry Crampton, Deerfield Academy.
Schaefer, Gertrude, Undergraduate, Temple University.
Seaman, Arlene, Zoology Assistant, Cornell University.
Snipes, Anne, Wheaton College.
Stees, Nancy, Teacher, West Chester State Teachers College.
Surrarrer, Thomas C., Professor of Biology, Baldwin-Wallace College.
Thornton, Dorothy Golden, Assistant in Zoology Dept., Wellesley College.
Tupper, Lyla, Graduate Student, Northwestern University.
Uber, Virginia M., Student, Pennsylvania College for Women.
Wax, Florence Sima, Student, Oberlin College.
Whyte, Marjorie Ann, Assistant, Cornell University.
Wilcox, Barbara L., Student, Radcliffe College.
Williams, Olwen, Teacher of Biology and Chemistry, The Putney School.
Wilson, Faith Evelyn, Johns Hopkins University.
Wilson, Marie Ellen, Student, Western Maryland College.

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4. Tabular View of Attendance

	1941	*1942*	*1943*	*1944*	*1945*
Investigators—Total	337	201	160	193	212
Independent	197	132	89	112	138
Under instruction	59	16	19	11	10
Library readers	31	28	35	50	38
Research assistants	50	25	17	20	26
Students—Total	131	74	68	75	96
Zoology	55	36	47	37	55
Embryology	37	24	13	23	23
Physiology	24	6	8	10	13
Botany	15	8	—	5	5
Total Attendance	468	275	228	276	308
Less persons registered as both students and investigators	7	2	6	1	
	461	273	222	275	
Institutions Represented—Total	144	126	116	106	124
By investigators	102	83	70	74	100
By students	72	43	41	41	49
Schools and Academies Represented					
By investigators	5	2	2	1	2
By students	2	—	1	2	2
Foreign Institutions Represented					
By investigators	3	—	2	2	1
By students	1	—	—	3	—

5. Subscribing and Cooperating Institutions

1945

Albany Medical College
Amherst College
Biological Institute, Philadelphia, Pennsylvania
Bowdoin College
Bryn Mawr College
Cathedral College
The Catholic University of America
Columbia University
Cornell University
Cornell University Medical College
Duke University
Fish and Wild Life Service, U. S. Dept. of the Interior
Fordham University
Goucher College
Harvard University
Harvard University Medical School
Industrial and Engineering Chemistry, of the American Chemical Society
Johns Hopkins University
Johns Hopkins Medical School
Lee Foundation
Eli Lilly & Company
Long Island University
Macy Foundation
Massachusetts Institute of Technology
McGill University
Miami University
Mount Holyoke College
New York University
New York University College of Medicine
New York University School of Dentistry
New York University Washington Square College
Oberlin College
Ohio State University
Pennsylvania College for Women
Princeton University
Radcliffe College
Rockefeller Institute for Medical Research
St. Joseph College for Women
Smith College
State University of Iowa
Syracuse University
Syracuse University Medical School
Temple University
University of Chicago
University of Connecticut
University of Illinois
University of Maryland Medical School
University of Michigan
University of Missouri
University of Pennsylvania
University of Pennsylvania School of Medicine
University of Rochester
Vanderbilt University Medical School
Vassar College
Washington University
Wayne University
Wellesley College
Wesleyan University
Western Maryland College
Western Reserve University
Wheaton College
Williams College
Wilson College
Woods Hole Oceanographic Institution
Yale University

6. Evening Lectures, 1945

Friday, June 29
Prof. P. W. Whiting .... "The Development of Hymenopteran Genetics."

Friday, July 6
Dr. R. R. Gates .... "Human Heredity in Relation to Animal Genetics."

Friday, July 13
Dr. I. Fankuchen .... "X-Ray Diffraction and Protein Structure."

Friday, July 20
Prof. S. C. Brooks .... "Our Interrelationships with South American Universities, together with Illustrated Travel Notes."

Friday, July 27
Prof. E. G. Butler .... "Problems of Differentiation and Dedifferentiation in Amputated Urodele Limbs."

Friday, August 3	
Dr. Bostwick H. Ketchum	"The Prevention of Ship Bottom Fouling."
Friday, August 10	
Dr. Daniel Merriman	"A Study in Pure and Applied Marine Biology. The Life History and Economic Importance of the Ocean Pout."
Friday, August 17	
Dr. Detlev W. Bronk	"Biological Research During the War and Postwar Periods."
Friday, August 24	
Dr. F. L. Hisaw	"Endocrines and the Evolution of Viviparity among the Vertebrates."
Monday, August 27	
George G. Lower	"Local Invertebrates."
Wednesday, August 29	
Dr. Paul S. Galtsoff	"Impressions of a Biologist at the San Francisco Conference."
Thursday, August 30	
Major A. H. Neufeld	"Medical Research Organization in the Canadian Army."
Thursday, August 30	
Capt. W. R. Duryee	"Medical Military Training."

7. Shorter Scientific Papers, 1945

Tuesday, July 24	
Dr. M. M. Brooks	"The Redox Potential of Penicillium rotatum Medium under Some Different Conditions of Growth."
Dr. Wilbur Robbie	"The Use of Cyanide in Manometric Experimentation."
Dr. Sears Crowell	"The Displacement of Terns by Gulls at Weepecket Island."
Tuesday, July 31	
Dr. P. W. Whiting	"The Problem of Reversal of Male Haploidy by Selection."
Dr. Berta Scharrer	"Experimental Tumors after Nerve Section in an Insect."
Dr. P. S. Galtsoff	"Reactions of Oysters to Free Chlorine."
Tuesday, August 7	
Dr. T. H. Bullock	"Organization of Giant Nerve Fibers in certain Polychaetes."
Dr. Ernst Scharrer	"The Origin of Neurosecretory Granules from Basophil Substances in the Nerve Cells of Fishes."
Dr. C. H. Taft	"The Action of Quitenine on the Livers of Tautog and Toadfish."
Dr. A. M. Shanes	"Evidence of a Metabolic Effect by Potassium in Lowering the Injury Potential of Nerve."

Tuesday, August 14

Dr. R. Chambers .... "Interrelations between Sperm-Nucleus, Egg-Nucleus and Cytoplasm in Asterias Egg."

Dr. Kurt G. Stern .... "Physical-chemical Studies on Chromosomal Nucleoproteins."

Tuesday, August 21

Dr. Dorothy Wrinch .... "Hemoglobin and other Native Proteins."

Dr. E. R. Witkus .... "Endomitosis in Plants."

Dr. C. A. Berger .... "Recent Cytological Studies in Culex."

Thursday, August 23

Dr. Ethel B. Harvey .... "Development of Granule-free Fractions of Arbacia eggs."

Dr. Alexander Sandow .... "Studies of the Muscle Twitch by Methods of Electronic Recording."

Dr. C. D. Beers .... "The Role of Bacteria in the Excystment of the Ciliate Didinium."

Monday, August 27

Dr. Anna R. Whiting .... "Differences in Sensitivity, Hatchability Curves and Cytological Effects between Eggs X-rayed in First Meiotic Prophase and Metaphase."

Dr. W. W. Wainio .... "Aerobic Oxidation of Simple sugars by Mammalian Liver."

Dr. Dugald E. S. Brown .... "The Role of Myosin and Myosin Triphosphatase *in Vitro* and in Muscle."

Tuesday, August 28

Dr. Lloyd M. Bertholf .... "Accelerating Metamorphosis in the Tunicate Styela."

Dr. Alfred Froelich .... "The Influence of Drugs on Heat-narcosis."

Dr. W. Malcolm Reid .... "*In Vivo* and *in Vitro* Glycogen Utilization in the Avial Nematode Ascardia Galli."

8. Members of the Corporation, 1945

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Moore, Dr. J. Percy, University of Pennsylvania, Philadelphia, Pa.
Morgan, Mrs. T. H., Pasadena, California.
Morgan, Prof. T. H., Director of Biological Laboratory, California Institute of Technology, Pasadena, California.
Noyes, Miss Eva J.
Porter, Dr. H. C., University of Pennsylvania, Philadelphia, Pennsylvania.
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Anderson, Dr. T. F., University of Pennsylvania, Philadelphia, Pennsylvania.
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Bartlett, Dr. James H., Department of Physics, University of Illinois, Urbana, Illinois.

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Bigelow, Prof. R. P., Massachusetts Institute of Technology, Cambridge, Massachusetts.

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Budington, Prof. R. A., Winter Park, Florida.

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THE INFLUENCE OF TEXTURE AND COMPOSITION OF SURFACE ON THE ATTACHMENT OF SEDENTARY MARINE ORGANISMS *

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Marine installations of various kinds necessitate exposure of construction materials under sea water. Data dealing with the amount of fouling accumulated by such materials are not abundant. Information which might be of aid to the scientist seeking the most favorable material upon which to collect sedentary organisms for study is also scanty. The present study was undertaken to determine the effect

TABLE I

Effect of surface texture of glass on attachment of sedentary organisms. (Numbers of individuals on each surface of 80 square inches of plate)

Surface number	Plain 0	Sand-blasted 1	Factrolite 2	Prestlite 3	Ribbed 4	Pentecor 5
Series No. 1, Tahiti Beach[1] 39 days (8/22/42–9/30/42)						
Hydroides sp.	143	265	152	506	349	197
Spirorbis sp.	85	188	122	90	163	110
Barnacles	1,948	1,072	1,162	975	1,674	2,140
Total	2,176	1,525	1,436	1,571	2,186	2,447
Average pop.	725.3	508.3	478.7	523.7	728.7	815.7
Average/square inch	9.1	6.4	6.0	6.5	9.1	10.2
Series No. 2, Miami Beach[2] 17 days (8/22/42–9/8/42)						
Wet weight (grams)	51.0	45.5	50.0	41.0	50.0	41.0
Dry weight (grams)	8.5	6.4	7.9	5.5	7.5	8.8
Barnacles	308	227	268	213	263	331
Series No. 3, Miami Beach[2] 30 days (9/15/42–10/15/42)						
Wet weight (grams)	164.5	174.0	149.0	150.0	126.0	155.5
Dry weight (grams)	51.5	50.0	30.0	24.0	24.0	33.0
Barnacles	642	515	554	778	798	977

[1] Subtropical testing service.

[2] Beach boat slips.

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* The observations described here were made while the authors were engaged by the Woods Hole Oceanographic Institution in an investigation of fouling, under contract with the Bureau of Ships, Navy Department, which has given permission for their publication. The opinions presented here are those of the authors and do not necessarily reflect the official opinion of the Navy Department or the naval service at large. Contribution No. 349 from the Woods Hole Oceanographic Institution.

of surface irregularities and of substrate composition on the establishment of sessile populations. The experiments were conducted in Biscayne Bay at Miami, Florida, where subtropical conditions favor the attachment of fouling organisms throughout the year.

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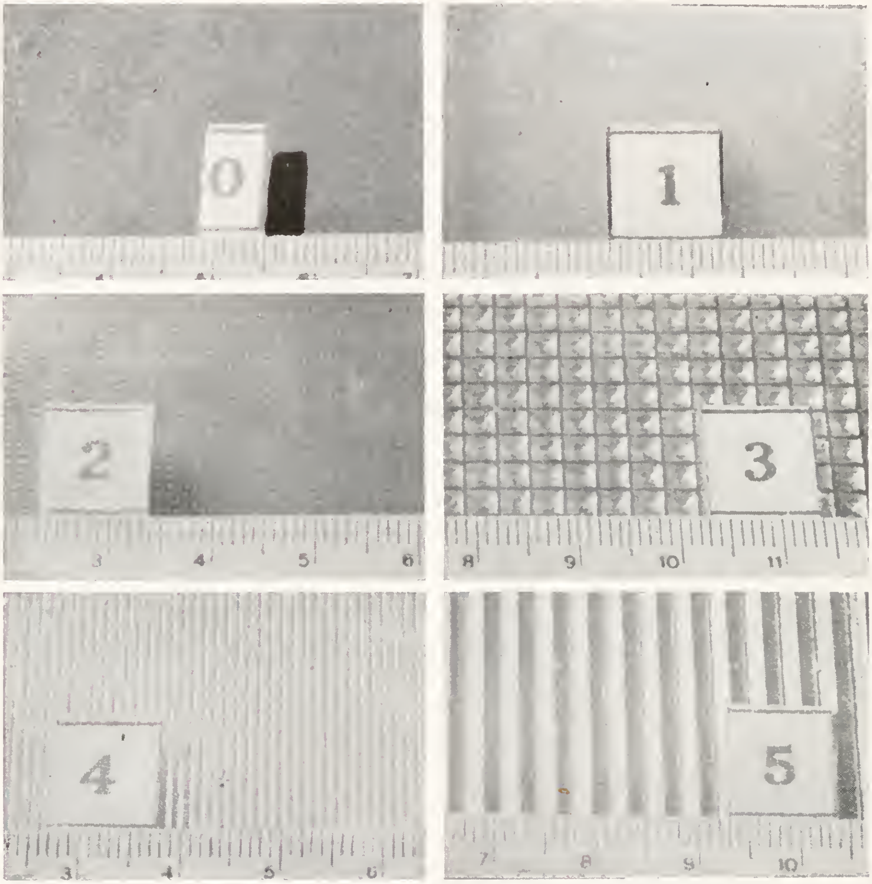

FIGURE 1. Glass surfaces used in testing the relation of surface irregularities to fouling. 0. Plain. 1. Sandblasted. 2. Factrolite. 3. Prestlite. 4. Ribbed. 5. Pentecor.

EFFECT OF SURFACE IRREGULARITY

Commercial glasses, manufactured by the Pittsburgh Glass Company, with various surface irregularities were used for this study. Six 8 × 10 inch glass plates were assembled, irregular surface down, in a rack suitable for floating on the surface of the water. The floats were constructed in such a way that sea water could move freely on both sides of the exposed surface. The backs of the panels which were all relatively smooth were placed upward. The fouling on the back surfaces was not recorded. The surface irregularities of the panels are shown in Figure 1 and may be described as follows:

Surface Number:

0. *Plain*	Smooth glass, polished.
1. *Sandblasted*	Glass sandblasted on lower side.
2. *Factrolite*	Surface consisted of pyramidal depressions of which there were about 144 per square centimeter.
3. *Prestlite*	Approximately nine pyramidal depressions per square centimeter.
4. *Ribbed*	Surface of V-shaped grooves, nine grooves per centimeter of width.
5. *Pentecor*	Approximately three V-shaped grooves per centimeter of width.

Results obtained from three series of experiments in which the glass surfaces were exposed are shown in Table I.

The Sessile populations which grew on the glass plates were composed primarily of barnacles and tubeworms, with irregular, perhaps seasonal, appearances of tunicates and *Anomia* sp. Barnacles (*B. improvisus* and *B. amphitrite niveus* in order of relative abundance) were numerous in both locations, but those at Tahiti Beach were always very small compared to those at Miami Beach. Many more barnacles attached to the lower (shaded) surfaces of the panels than to the upper surfaces where light was more abundant. This is in agreement with the experience of Pomerat and Reiner (1942), who report that larger numbers of barnacles accumulate on dark surfaces than on light surfaces. The shaded undersides of the glass panels, being darker than the upper sides, appear to attract more cyprids and hence show a greater barnacle accumulation.

The various surface textures of glass had little influence on the number of attached organisms. In these experiments barnacles were consistently slightly more numerous on Pentecor than on smooth glass. This behavior was confirmed in the experiment reported in the following section although conditions of exposure were not exactly parallel. In the first experiment the glass panels were floated at the surface in a shaded location, while in the second they hung vertically below low tide

TABLE II

Influence of substrate on fouling, sixty days' exposure at the beach boat slips, September 25, 1942–November 25, 1942

Substrate	Weight of fouling on panel area of 264 sq. in., that of wood panels employed	
	Wet weight grams	Dry wt. grams
1. Dade County pine	675.1	346.5
2. Gum	1127.6	531.4
3. Magnolia	1165.4	446.4
4. White pine	968.7	446.8
5. Cypress	954.8	392.0
6. Tile	980.1*	487.3
7. Cement	1033.0*	534.1
8. Glass	386.1	167.3

* Corrected to an area, 264 sq. in., equal to that of the wood panels.

under sun exposure. Counts of tubeworms were made on only one set of exposures. *Hydroides* sp. was most abundant on Prestlite and *Spirorbis* sp. was most abundant on sandblasted glass.

Composition of the Surface

Unpainted panels of wood of five species, clay roofing tiles, cement roofing plates, and a glass panel were exposed for 60 days at the Beach Boat Slips in Miami Beach.

Table III

Effect of substrate on fouling, exposures of three months at South Dock, Belle Isle, Miami Beach, Florida, January 9, 1943–April 9, 1943, all materials applied to, or mounted on, wood unless otherwise noted

Composition of surfaces	Wet weight* (grams)	Dry weight* (grams)	Number* of barnacles	Notes
Plastics				
1. Celluloid	3.8	2.2	11	Thin coat of algae.
2. Plasticel	24.3	12.2	124	Barnacles' bases easily removed.
3. Lucite	5.6	1.7	41	
4. Formica	6.9	3.2	11	
5. Isobutyl Methacrylate	15.4	7.2	70	Film applied to glass panel. Plastic peels intact with barnacles.
Glass				
6. Prestlite	57.0	25.2	176	Some barnacles 12 mm. across.
7. Pentecor	46.0	25.0	148	Some barnacles 12 mm. across.
8. Sandblasted	23.6	7.0	46	6 calcareous tubeworms; tunicates.
9. Smooth	4.5	1.7	16	Green slime may have caused fish to remove young barnacles.
*Paints and ingredients***				
Coatings applied to steel panels				
10. Ester gum vehicle	36.3	8.1	58	Tunicates and bryozoa.
11. Rosin vehicle	2.7	0.4	0	Fish spawn both sides.
12. Anticorrosive paint 42-A	2.7	0.5	9	Baracles very small.
13. Vehicle of 15RC	6.1	3.3	43	
14. Antifouling paint 7C	0.0	0.0	0	Some slime film.
15. Antifouling paint 8C	0.6	0.3	14	Small barnacles close to edge.
Coatings Applied to Wood				
16. Ceraloid	57.6	38.5	183	
17. Paraffin	11.3	6.1	59	Lomnoria active in breaking paraffin.
18. Asphaltum	121.4	34.3	768	Barnacles only.
19. Asphaltum varnish	67.8	13.8	256	Some bryozoa.
20. Spar varnish	45.1	7.0	304	
21. Navy grey	41.6	5.6	150	Algae.
22. Anti-corrosive 42-A	48.2	10.7	156	

* Corrected to an area of 144 square inches.

** Anticorrosive 42A is a standard Navy formula. Vehicle of 15RC is the non-pigmented portion of a standard Navy antifouling paint. Antifouling paints 7C and 8C are experimental modifications of a standard Navy antifouling paint of the cold plastic type in which the toxic pigment is reduced to 50 and 60 percent of the normal value.

TABLE III—*Continued*

Composition of surfaces	Wet weight* (grams)	Dry weight* (grams)	Number* of barnacles	Notes
Woods				
23. Dade County pine (soaked 60 days)	395.2	120.7	748	Bryozoa.
24. Gum (soaked 60 days)	452.1	133.4	686	
25. Dade County pine (unsoaked)	144.3	27.3	125	Hydrozoa, bryozoa.
26. Gum (unsoaked)	249.8	43.5	222	
27. Soft pine	57.6	11.5	184	
28. Teak	143.8	88.7	306	Large barnacles.
29. Maderia	173.7	84.2	358	Many fish eggs.
30. Greenheart	77.0	40.8	342	
31. Balsa	2.9	1.6	5	Wood very soft.
Metals				
32. Steel	224.4	42.8	88	
33. Galvanized iron	2.6	0.7	6	Barnacles easily removed.
34. Zinc	1.0	0.2	0	Active corrosion.
35. Lead	30.6	50.9	396	Large barnacles.
36. Monel	1.6	0.5	6	Many fish eggs.
37. Nickel	43.2	10.7	126	
38. Galvanized iron pipe	4.7	3.0	27	Barnacle on rusted threads and damaged edges.
Miscellaneous				
39. Linoleum	79.7	23.0	193	
40. Deck canvas no. 10	5.1	2.3	7	Sagging-algae eaten by fish.
41. Sole leather	32.4	12.4	66	
42. Masonite, heat tempered	138.6	31.8	594	Brown tunicates.
43. Asbestos	284.2	65.9	980	Bryozoa, *Anomia*, hydrozoa, calcareous tubeworm.

All panels were suspended in a vertical position approximately two feet beneath the mean low water mark. The site was well shaded by a protecting roof. The results obtained are presented in Table II.

The weights of the populations (barnacles, tubeworms, tunicates, bryozoa, and algae) which accumulated on the woods, tile, and cement were of the same order of magnitude, though variations as great as 59 per cent were observed. The weight of organisms accumulated on glass was approximately 30 per cent of that collected from other substrate materials.

A much larger number of materials were tested in a second experiment, the results of which are given in Table III. Exposure was made for three months at South Dock, Belle Isle, in Miami Beach, where conditions of bright sunlight, active current movement, and moderate fouling incidence were found. Growth on the panels consisted primarily of barnacles (*B. improvisus* and *B. amphitrite niveus*) with occasional tufts of hydrozoa and patches of colonial tunicates. A blanket of algae having very short filaments grew on panels of light color or shaded background. Large sets of fish eggs were found on the rosin vehicle and Monel. Borings of *Limnoria* sp. were everywhere evident in unprotected wood.

The substrates accumulating heaviest populations were asbestos, asphaltum, Dade County pine (pre-soaked 60 days), gum wood (pre-soaked 60 days) and Masonite. Asbestos shingle, commonly used as clapboarding, yielded the richest harvest as measured by the number of barnacles. A comparison of asbestos and Masonite, two of the best collectors, is shown in Figure 2. The asphaltum used was of the type employed as aquarium cement. It accumulated barnacles only.

Panels of gum and Dade County pine, which had been exposed for 60 days in the earlier test reported in Table II, were included for comparison with unsoaked specimens of these woods. The unsoaked woods developed much less fouling.

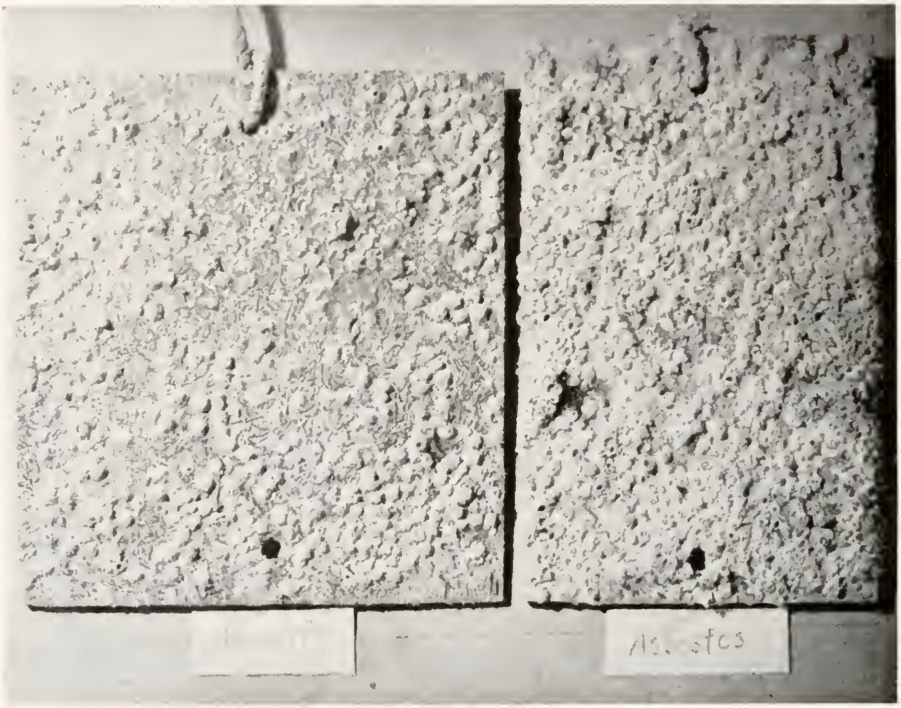

FIGURE 2. Accumulation of fouling organisms on masonite and asbestos after 90 days' exposure at Belle Isle, Miami Beach, Florida.

Intact galvanizing on iron was very resistant to marine life. No barnacles were obtained on zinc, on the experimental antifouling paint 7C or on rosin vehicle, a common paint component.

Materials with hard non-porous and non-fibrous surfaces were in general rather poor collectors of fouling. The best accumulation of sedentary populations was found on surfaces which were porous and/or fibrous. Surface of paints, paint ingredients and linoleum are in general non-porous and non-fibrous. Compared to the size and strength of the barnacle cyprid they are also smooth and hard. The histogram (Fig. 3) summarizes the collective efficiency of the substrates.

Some results were undoubtedly spurious, and these should be noted. Fouling on the antifouling paint 8C which occurred along the edges of the panel was probably

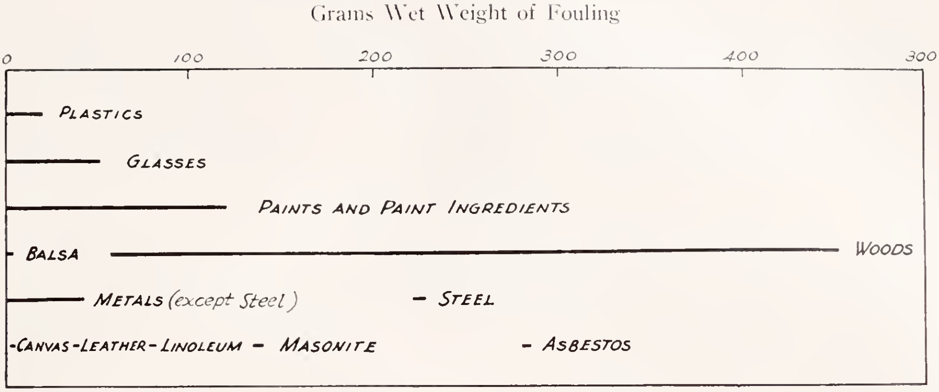

Figure 3. Relative amounts of fouling on various classes of materials used as test panels.

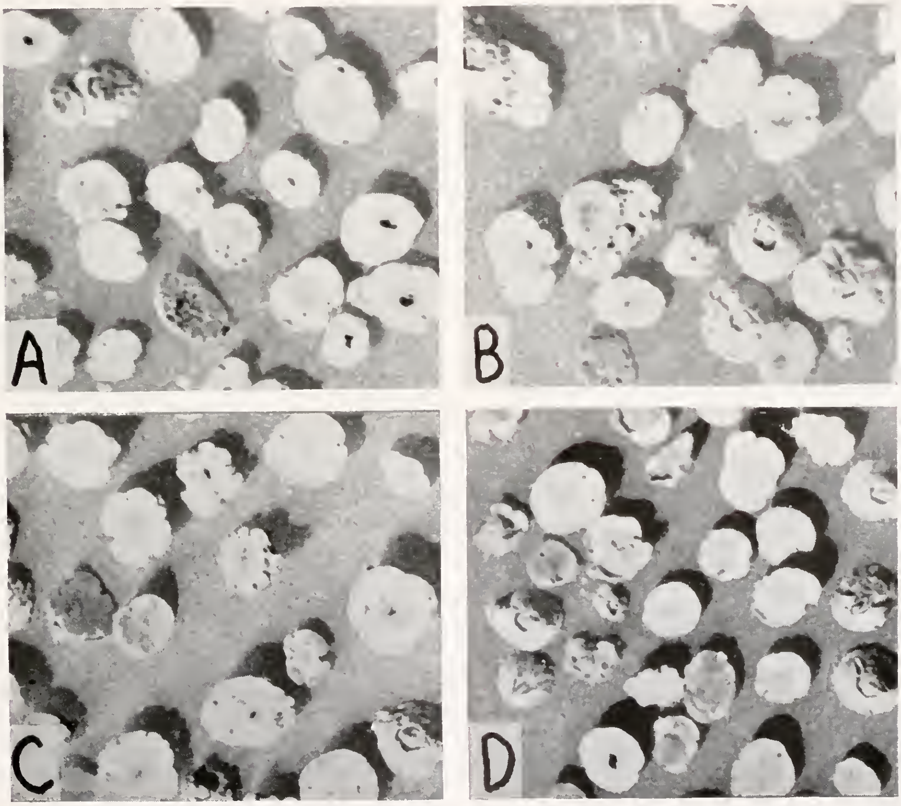

Figure 4. Bases of barnacles grown on various substrates. A. Navy grey. B. Antifouling paint 15RC. C. Ester gum. D. Anticorrosive paint 42A.

due to imperfections of the paint surface. In contrast, 7C which contained less copper was not fouled. Deck canvas and smooth glass both supported a culture of green algae which evidently served as food for fish. Active feeding on these panels unquestionably disturbed other fouling organisms. Balsa wood was apparently sloughing its surface and thus loosening attached forms. In spite of these minor qualifications, the results involve a range of population numbers sufficiently wide to indicate the relative merits of the substrates used.

One of the most interesting of the results was observed when barnacles were removed from the various substrates. Some of the substrates bore barnacles with deeply scalloped margins (Fig. 4) instead of the typical smooth edges. These margins suggested that localized irregular marginal growth interruptions had taken place. Such barnacles were collected from:

Spar varnish
Linoleum
Navy grey topside paint (P–50)
Antifouling paint vehicle 15RC
Ester gum paint vehicle
Anticorrosive paint 42–A

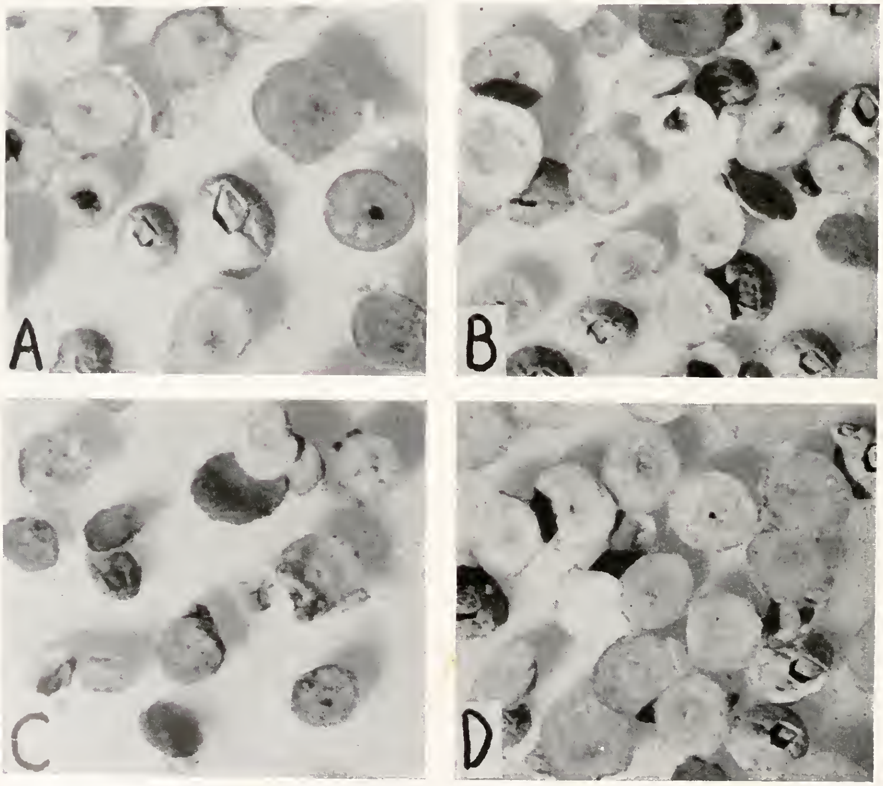

FIGURE 5. Bases of barnacles grown on various substrates. A. Isobutyl methacrylate. B. Plasticel. C. Soft paraffin. D. Ceraloid.

Barnacles growing on soft paraffin had distinctly concave bases. Mosaics of bases with angular margins were typical of barnacles attached to lead but were also found on other overcrowded substrates. It was possible to remove barnacles with intact bases very easily from several materials, including plasticel, ceraloid, and isobutyl methacrylate (Fig. 5). This finding might prove useful in designing experiments in which the minute anatomy of basal structures was to be studied.

SUMMARY

1. Submerged samples of 40 different construction materials were used as substrates for the collection of sedentary populations. The barnacle counts in the populations ranged from 980 on asbestos shingles to zero on zinc and on two paint coatings, after three months' immersion in Biscayne Bay at Miami Beach, Florida.
2. Various surface textures of glass plates were found to exert no significant influence on the accumulation and growth of sedentary marine organisms, although smooth clear glass accumulated smaller populations in the comparatively short exposure periods, 1–3 months .
3. The results suggest that efficiency of a substrate as a fouling collector is in general correlated with porosity of surface or with fibrous nature of surface. Smooth, non-porous, non-fibrous surfaces, especially if also hard, seem to be poor accumulators of sedentary organisms.
4. Further testing of substrates is greatly to be desired in this connection.

REFERENCES

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THE DEVELOPMENTAL HISTORY OF AMAROECIUM CONSTELLATUM. II. ORGANOGENESIS OF THE LARVAL ACTION SYSTEM

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INTRODUCTION

The early development of the embryo of *Amaroecium constellatum* has been presented in a previous paper (Scott, 1945). The accumulation of yolk modifies the pattern of mosaic development characteristic of Tunicates to the extent that gastrulation is accomplished in an atypical manner. Convergence of the cells of the lateral margins of the posterior blastoporal lip is accomplished to the left of the mid-line. The neural plate elongates posteriorly at the place where the lateral blastoporal lips meet and close. The chordal cells are inflected at the anterior lip and lie in the median axis. The potential muscle cells of the morphological right side lie dorsal to the notochord as a result of their growth across the mid-dorsal plane, the muscle cells of the morphological left side lie below the level of the notochord on the curved left side of the embryo. The two groups of muscle cells are separated by the posterior extension of the neural plate.

MATERIALS AND METHODS

Amaroecium constellatum is abundant along the eastern coast of the United States. The breeding season lasts throughout the summer months. Material for this study was collected at Woods Hole, Massachusetts. The embryos, squeezed from adult colonies, were selected and arranged into a progressive series of stages for study. They were fixed in Bouin's fluid. Some were stained by Conklin's modification of Delafield's haematoxylin, others with borax-carmine, then cleared according to the benzyl-benzoate method described in Romeis' "Taschenbuch der Mikroscopischen Technik." Corresponding stages were sectioned serially, stained in Mayer's or Gallagher's or iron haematoxylin, and counterstained with eosin or triosin. All drawings were made with the aid of a camera lucida. The photomicrographs were made with a Leitz "Macca" camera using Zeiss apochromat, 20 ×, and fluorite oil immersion, 100 ×, objectives with a Zeiss microscope.

Later embryonic development

It seems advisable to present a descriptive series of developmental stages that may be used as points of reference for structures differentiating during the organ-forming period. For convenience the developmental period following gastrulation is divided into four stages; 1) the tail bud stage, 2) early tadpole stage, 3) prehatching stage, and 4) the free-swimming tadpole stage. The free-swimming larva or tadpole has been described thoroughly by Grave (1921) and shall be presented here in brief summary since reference to it is necessary. A short description of the external appearance of these stages will be given first and referred to in subsequent treatment of organogenesis as Stages I, II, III, and IV. The terms, larval action system and adult action system, used by Grave (1935, 1944) will be adopted for the structures functioning during larval life and those functioning during adult life respectively.

The tail bud stage

By the end of gastrulation the embryo is approximately spherical except for a shallow postero-ventral invagination of the ectoderm constricting tail from trunk region. The furrow appearing first on the right side is deeper there, and less deep as it extends to the left side. The tail bud is short and rounded, curving immediately toward the ventral side of the trunk. Through the thin epidermis quadruple rows of large muscle cells can be seen lying dorsal and ventral to the notochord. The neural plate is elevated at the periphery to form the neural groove, enclosing anteriorly a wide depression, the presumptive brain region, posteriorly a narrow, trough-like depression lying to the left of the notochord, the presumptive neural tube (Fig. 1*A*).

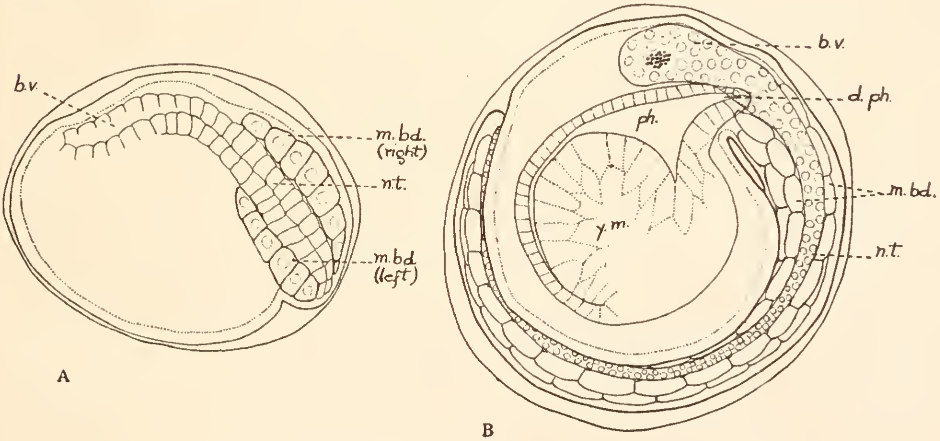

FIGURE 1. *A*. Stage I, embryo before neural folds close. 160 ×. *B*. Stage II, early tadpole; beginning of differentiation of digestive and nervous systems. 160 ×. *b. v.*, brain vesicle; *d. ph.*, dorsal diverticulum of pharynx; *m. bd.*, muscle band; *n. t.*, neural tube; *ph.*, pharynx; *y. m.*, yolk mass.

A transverse section through the tail bud stage discloses that the embryo is solid. A single layer of definitive endoderm lies under the concave neural plate (Fig. 5*E*). This layer of cells develops from the cells that form the superficial "pseudo-invagination" cavity of gastrulation. The depression closes by a reversal in change of shape of the cells involved rather than by approximation of the lips of the blastopore thus producing a solid archenteron (Scott, 1945). The endodermal cells spread under and anterior to the neural plate. Ventral to them is located the mass of heavily yolk-laden cells derived from the macromeres. Wedged between the thin ectoderm and the solid endoderm on either side is a mass of mesenchyme, small, polygonal cells with prominent nuclei (Fig. 5*E*).

Posteriorly the definitive endoderm lies adjacent to the chordal cells which are beginning to interdigitate in the base of the tail bud. The mesenchyme terminates abruptly in this region against the muscle cells of the tail.

Early tadpole stage

The embryo increases in size and acquires the shape that justifies its being called "tadpole." The trunk region elongates slightly in the antero-posterior axis remaining curved at the anterior end. The tail encircles the body meridionally as it grows in length. The embryo is still opaque.

The neural folds are closed, the position of the sensory vesicle being marked by aggregations of black pigment which show through the surface of the body. The neural tube is faintly visible along the side of the tail. More conspicuous are the large muscle cells dorsal and ventral to the prominent notochord which forms the axis of the tail throughout its length. Dorsally, on either side of the sensory vesicle there is a slight ectodermal invagination, rudiments of the atrial chambers. The embryo is confined within a test the cells of which are arranged in a compact layer (Fig. 1*B*).

Pre-hatching stage

Changes in the external appearance of the later embryo depend on the development of siphons and adhesive papillae and the secretion of a tunic. As body growth continues and organs of the larval action system differentiate, the body becomes transparent except where the mass of yolk is lodged in the pharynx.

The trunk region continues to elongate antero-posteriorly becoming elliptical in shape. Posteriorly the body narrows to the base of the tail; anteriorly it flares in the dorso-ventral axis in relation to the vertical position of the adhesive papillae. Laterally the body is compressed. A thickening layer of tunic invests the entire trunk. It is indented at the junction of trunk and tail and continues over the surface of the tail. The tail encircles the body meridionally being pressed into a groove in the tunic. The tunic of the tail projects laterally into fins.

The sensory vesicle occupies a dorsal position at the posterior end of the trunk. Two masses of pigment project into its cavity. Immediately in front of it lies the elevation of the oral siphon; behind it and on the posterior curve of the body lies the atrial siphon. Much of the internal structure is visible through the tunic and mantle. The incipient adhesive papillae appear as three disc-like projections in verticle series at the rounded anterior end (Fig. 2).

The free-swimming tadpole stage

The trunk of the tadpole of Amaroecium at its release measures about 600 micra in length; it measures about 270 micra in depth. The tubular atria with their triple rows of gill slits are pressed into the dorsal pharynx through half of its length posteriorly. An obvious structure in the pharynx is the dorsal, heavily ridged endostyle which seems to rest on the lateral masses of yolk that form the wall of the pharynx. The transparent pericardium occupies a large space below the yolk anteriorly in front of the loop of alimentary tract. On the right side of the body the stomach extends along the posterior and ventral curvature of the yolk. On the left the narrow intestine curves along the side of the stomach up to the left atrium where it terminates. The root of the tail lies in the posterior third of the length of the body.

Anteriorly, the adhesive papillae project into the tunic in a vertical row slightly to the right of the median plane. The test vesicles lie loosely within the tunic or many of them, even at the time of hatching, retain a slender connection with the cone or ridge from which they originate. Where the tail is continuous with the trunk the tunic dips down into an abrupt pocket. The epidermis secretes a thin sheath of tunic about the tail. Laterally it expands into wide sail-like fins (Fig. 3).

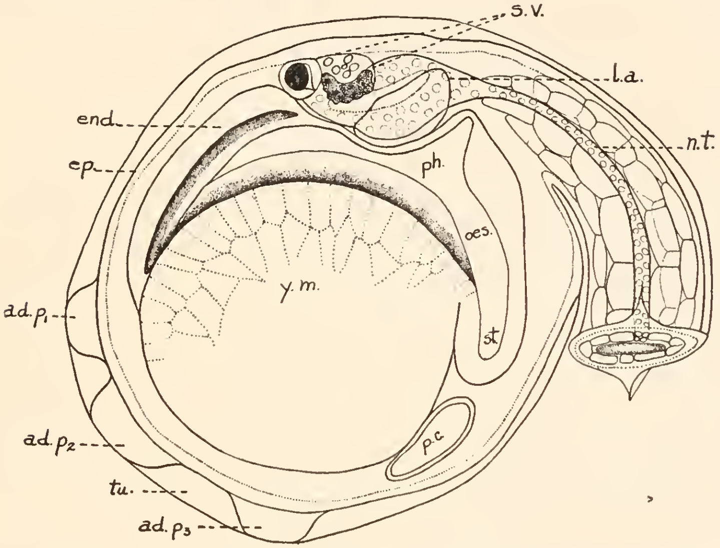

FIGURE 2. Stage III, lateral view of tadpole with incipient adhesive papillae. About 120 ×. *ad. p.*, adhesive papillae; *end.*, endostyle; *ep.*, epidermis; *l. a.*, left atrium; *n. t.*, neural tube; *oes.*, oesophagus; *p. c.*, pericardial cavity; *ph.*, pharynx; *st.*, stomach-intestine rudiment; *s. v.*, sensory vesicle; *tu.*, tunic; *y. m.*, yolk mass.

ORGANOGENESIS OF THE LARVAL ACTION SYSTEM

Digestive system

The pharyngeal cavity develops in Stage II by delamination between the layer of definitive endoderm and the mass of yolk-laden cells, appearing first below the brain and spreading from that point (Fig. 1*B*, 5*F*, 6*E*). It extends back to the base of the notochord as an upwardly directed diverticulum. Ventral to the base of this projection a second invagination appears, the rudiment of the stomach and intestine located a little to the right of the median plane on the inner side of the visceral ganglion (Fig. 2, 6*F*).

The pharynx deepens in Stage III encroaching upon the mass of yolk cells. Gradually thin septa of epithelium divide the yolk mass into four compact longitudinal columns, the two on each side being continuous at the bottom. The central

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two are lower than the outer two, thus providing greater depth for the limited pharyngeal cavity (Fig. 6*A, F*). This supply of nutritive material in the pharynx remains to be digested during the active life of the larva and throughout the critical period of metamorphosis. All other tissues lose their meager supply of yolk almost entirely, leaving their cytoplasm clear.

Along the roof of the pharynx, anterior to the place of origin of the oral siphon, the epithelium rises up into a double fold enclosing the endostyle, restricted to the dorsal side above and between the lateral masses of yolk and passing to the anterior end of the yolk mass (Fig. 2, 6*A*). Before the tadpole is released from its test, the cells in the floor of the groove develop long cilia. The pharynx grows out above and below the atrial sacs, bringing the mesial atrial and lateral pharyngeal walls into intimate contact (Fig. 6*B*).

Due to the combined activity of atrial and pharyngeal epithelia, three horizontal rows of gill slits are formed, each consisting of seven or eight perforations. The bordering cells of each gill develop a heavy brush of cilia, precocious equipment from the functional point of view. Even though the mouth breaks through to the branchial chamber, the tunic fills up the oral and atrial siphons until metamorphosis is completed.

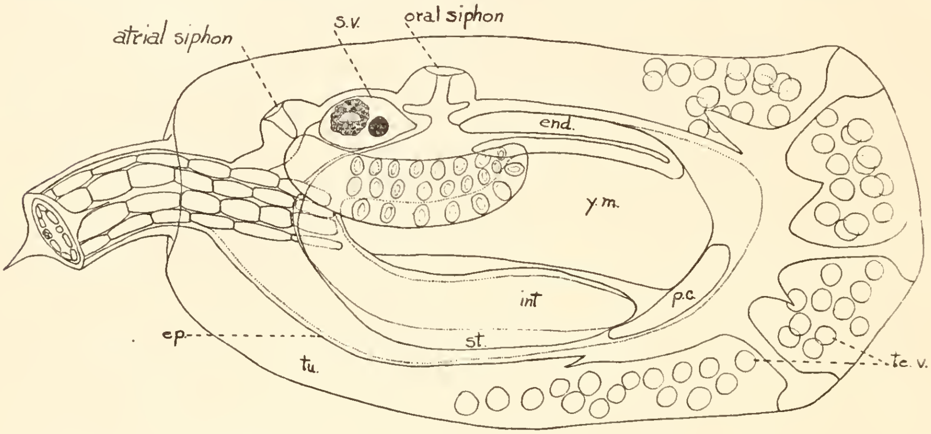


FIGURE 3. Stage IV, tadpole at hatching. About 120 ×. *end.*, endostyle; *ep.*, epidermis; *int.*, intestine; *p. c.*, pericardial cavity; *st.*, stomach; *s. v.*, sensory vesicle; *te. v.*, test vesicles; *tu.*, tunic; *y. m.*, yolk mass.

The rudiment of the oesophagus grows forward along the curvature of the yolk and dilates to form the stomach. The diverticulum extends to the midventral region of yolk where it turns sharply upon itself and continues backward as the slender intestine. With a gradual slope upward the intestine retraces the course of the stomach on its left side terminating ventral to the posterior end of the left atrium (Fig. 3, 6*B*). Later the anus opens into the atrium here.

There are no cilia evident in the intestine or stomach during this period of development. The wall of the stomach is thicker than the wall of the remaining parts of the digestive tract although the alimentary epithelium, throughout its length, consists of a single layer of cells.

With rapid general growth of the body, the loop of intestine and stomach increases in length anteriorly, extending through the posterior half of the body cavity below and behind the yolk-laden pharynx (Fig. 3). The pericardium lies directly in front of it. Between the arms of the loop posteriorly are lodged the bases of the axial organs of the tail.

Atrium—During Stage II the atrium or peribranchial sac appears as a pair of ectodermal invaginations, one on either side of the sensory vesicle (Fig. *6E*). At the place of its origin the neck of each depression constricts and separates from the surface.

In the transition from Stage II to Stage III, the atria, in contact with the lateral endodermal wall of the pharynx, grow in an anterior direction only, with the result that the atrial chambers are horizontal capsular cavities located dorsally, one on either side of the pharynx (Fig. 6B). They extend through the posterior two-thirds of the trunk, curving gently upward posteriorly where they grow towards each other and unite behind the pharynx (Fig. 3). The atrial siphon opens through the dorsal wall of this connecting canal between the two cavities.

The atrial walls are characteristically thin and the cells lose their intercell membranes. Occasional yolk granules are scattered through the cytoplasm. During Stage III the gill slits perforate the walls in three horizontal rows on the inner side in direct contact with the wall of the pharynx. The lowermost row develops first, the atrial and pharyngeal fusing first in these regions. The slits number between seven and nine in each row. Later in the free-swimming period the cells bordering the gill aperture produce long cilia. The endoderm has no part in atrial formation except insofar as the gill slits are the product of joint activity of atrial and pharyngeal walls (Caullery, 1895).

Oral and Atrial Siphons—Late in Stage III the dorsal ectoderm in front of the sensory vesicle thickens and invaginates, pushing the endoderm of the pharynx before it. The circle of epidermis around the invaginated area becomes elevated, giving the oral siphon a crater-like appearance (Fig. *6B*). The floor of the invagination thins out in a flat layer against the pharyngeal roof with which it is in contact. The lower part of the cavity projects outward from the center and produces a ring-shaped extension on the mouth opening. The oral cavity assumes the shape of a flask with a long neck and a flattened base (Fig. 3). Into this ectodermal cavity, or stomodaeum, the hypophysial duct opens, just before hatching of the tadpole. Although the oral plate breaks through late in the tadpole's development, the tunic fills up the stomodaeal portion and prevents the passage of both food and water during larval life.

The atrial siphon, like the oral, is formed by ectodermal invagination. The thickened mantle is elevated, raising the siphon above the level of the rest of the mantle in knob-like fashion (Fig. *6C*). The floor of the invagination fuses with the dorsal wall of the connecting arm of the atrium. The atrial siphon is situated on the downward curve of the dorsal surface just posterior to the sensory vesicle and anterior to the insertion of the tail (Fig. 3, *6G*, *H*). The epithelial lining of the oral and atrial siphons projects into each opening at several points forming small tentacles. The mesenchymatous muscles in the mantle in this region provide the contractile elements that control the apertures when the siphons begin to function.

Heart and pericardium

Towards the end of Stage III, the endodermal cells extend completely around the yolk mass as a definite epithelium. Mid-ventrally it evaginates into the body space and constricts off from the yolk epithelium. The bladder-like vesicle is the pericardium which invaginates mid-dorsally into an inner enclosed vesicle, the heart. The cells lose their inter-cell membranes and the nuclei bulge irregularly in both cardial and pericardial walls (Fig. 6*A*). The heart does not develop beyond this point at present, the circulatory system not functioning during larval life.

The nervous system

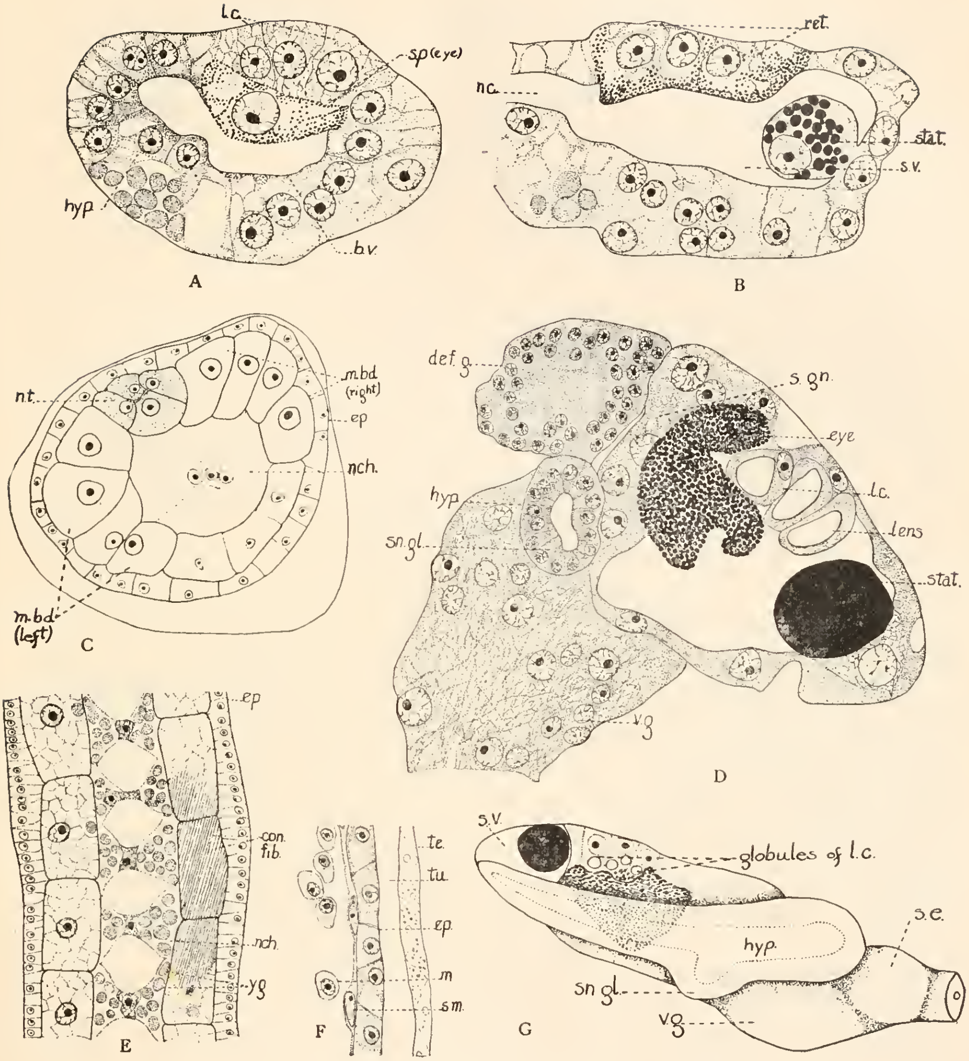
The neural folds of Stage I close in the early phase of Stage II thus forming the hollow nervous system typical of chordates except in one point, the curving of the neural tube through 90° to the left of the brain region. The anterior portion of the nervous system produces the sensory vesicle with its sensory organs, the hypophysis, definitive ganglion, and the so-called subneural gland. The intermediate part including the origin of curvature and a small contribution from the brain region gives rise to the visceral ganglion and the spinal enlargement, the posterior part becomes the neural tube.

The cavity of the brain region is slightly dilated and its wall uniformly thick. The neural tube consists, in section, of four cuboidal cells surrounding a small lumen (Fig. 4*C*). Cell membranes in both regions are distinct at this stage, the nuclei are large and contain heavily staining nucleoli. The cytoplasm is reticular in appearance and has occasional yolk granules.

During Stage III the brain vesicle differentiates into two structures, the sensory vesicle in the entire right side and the rudiment of the hypophysis on the left posterior side (Fig. 4*A*, 5*A*). The vesicle expands, its walls becoming thin; the rudiment of the hypophysis remains small with thick walls. This secondary cavity is separated completely from the sensory vesicle at the region of evagination but their walls remain attached throughout subsequent development (Fig. 5*B*, *C*).

The sensory vesicle—Two sensory structures develop in the sensory vesicle, the statolith and the eye. The left posterior wall of the vesicle thickens, the right wall expands dorsally and laterally; all the cells lose their inter-cell membranes. The left wall of the cavity remains thick and constitutes the sensory ganglion of the brain. One cell on the ventro-anterior wall projects into the cavity and large pigment granules are deposited in its cytoplasm; these coalesce to form the statolith (Fig. 4*B*, 5*B*). In Stage IV the statolith is a spherical mass of pigment confined within the cell membrane and attached to the ganglionic wall by a stout stalk, the remaining part of the cell (Fig. 4*D*, 5*C*, *D*).

A group of cells situated dorso-laterally at the left posterior limit of the vesicle initiates the development of the eye by the deposition of pigment granules of much smaller size than those that form the statolith. Absence of cell membranes makes it difficult to ascertain the number of cells that participate in this activity. The pigment is deposited in the shape of a cup, its concavity facing dorso-laterally and to the right within the vesicle. Three ganglionic cells which retain their membranes fill up the concavity in series. They secrete globules of liquid which increase in size both by the gradual addition of the secretion and by the fusion of globules. The globules of liquid form the so-called lens cell (Fig. 4*A*, *B*, *D*, 5*D*). The nuclei


l.c.
sp (eye)
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A
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m.bd (left)
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FIGURE 4. *A*. Transverse section through brain after the neural folds close, Stage II. 750 ×. *B*. Longitudinal section through brain of same stage. 750 ×. *C*. Cross section through tail of tadpole, Stage III. 300 ×. *D*. Section through brain of tadpole just before hatching, oblique to include both sensory organs. 750 ×. *E*. Longitudinal section through tail of tadpole in Stage III. 300 ×. *F*. Section through epidermis and test of Stage III. 750 ×. *G*. Reconstruction of brain and related structures of Stage III, viewed from left side. 300 ×. *b. v.*, brain vesicle; *con. fib.*, contractile fibrils; *def. g.*, definitive ganglion; *ep.*, epidermis; *hyp.*, hypophysis; *l. c.*, lens cell; *m. bd.*, muscle band; *n. c.*, neural canal; *nch.*, notochord; *n. t.*, neural tube; *ret.*, retinal cells of eye; *s. gn.*, sensory ganglion; *sn. gl.*, subneural gland; *stat.*, statolith; *s. c.*, sensory cell; *s. e.*, spinal enlargement; *s. m.*, smooth muscle cells of mantle; *s. p.*, sensory pigment; *t. c.*, test cells; *tu.*, tunic; *v. g.*, visceral ganglion; *y. g.*, yolk granules.

which at first occupy a central position in the cells are pushed to the periphery as the lenses, increasing in size, come eventually to monopolize the entire cell.

The pigment granules of the eye always remain discrete, not coalescing as do those of the otolith. Extending through the concentrated pigment are small rods of clear cytoplasm. They run from the base of the cup back towards the ganglion. Seven or eight of them may be seen in embryos of Stage IV that are mounted in a mixture of benzyl-benzoate and oil of wintergreen.

The hypophysis—The rudiment of the hypophysis early in Stage III appears as an extension or small evagination of the brain cavity (Fig. 4*A*, 5*A*). The cells retain their membranes, their nuclei are smaller than those of the adjoining part of the brain. Histologically they present the appearance of epithelial tissue. Upon its separation from the primary cavity during Stage III it elongates antero-posteriorly along the left side of the sensory ganglion (Fig. 4*G*). In Stage IV it ends blindly at the posterior wall of the oral siphon. Later these walls fuse and the hypophysis communicates with the posterior region of the stomodaeum, extending along the side of the ganglion with a gentle slope upward as far as the atrial siphon where it terminates blindly. The floor of the duct, corresponding in position to the region of the eye, deepens abruptly (Fig. 4*D*, *G*). The ventral wall of the pocket becomes slightly thicker, the indentation with its thickened floor constituting the subneural gland. Hjort (1896) reviews the opposing views concerning this structure in the early works on Tunicates.

The definitive ganglion—By a proliferation of cells in the mid-region of its roof in Stage II the hypophysial duct produces an oval mass containing small nuclei similar to those in the hypophysial duct itself. The cell membranes disappear and the nuclei wander out toward the periphery where they collect in several rows with the granular cytoplasm concentrated in the center (Fig. 4*D*, 4*G*, 5*C*). This part of the nervous system, the definitive ganglion, persists through metamorphosis and together with the hypophysis gives rise to the permanent nervous system of the adult.

Visceral ganglion—The visceral ganglion originates in that part of the neural plate that curves toward the left in Stage I. The lumen is obliterated, the large nuclei migrate to the periphery leaving the medulla mass of interlacing fibrils and granules (Fig. 4*D*). The visceral ganglion lies posterior to and ventral to the sensory vesicle. Dorsally where it merges with the sensory ganglion, it exceeds the sensory vesicle in diameter but it gradually diminishes in diameter towards the base of the tail where it is continuous with the neural tube. At the junction there is a slight enlargement called the spinal enlargement (Fig. 4*G*). The neural tube retains its lumen. It runs through the length of the tail to the left of the notochord. In Stage IV a single nerve emerges from the visceral ganglion on its right side just below the hypophysis. It runs anteriorly and sends out branches to the smooth musculature of the mantle.

The Notochord

At the end of the gastrulation period the chordal cells lie under the posterior part of the neural plate. Anteriorly adjacent to them are endodermal cells; dorsally, the potential muscle cells of the right lateral margin of the blastopore; ventro-laterally, the potential muscle cells of the left lateral margin of the blastopore. Posteriorly the chordal cells extend into the rudiment of the tail.

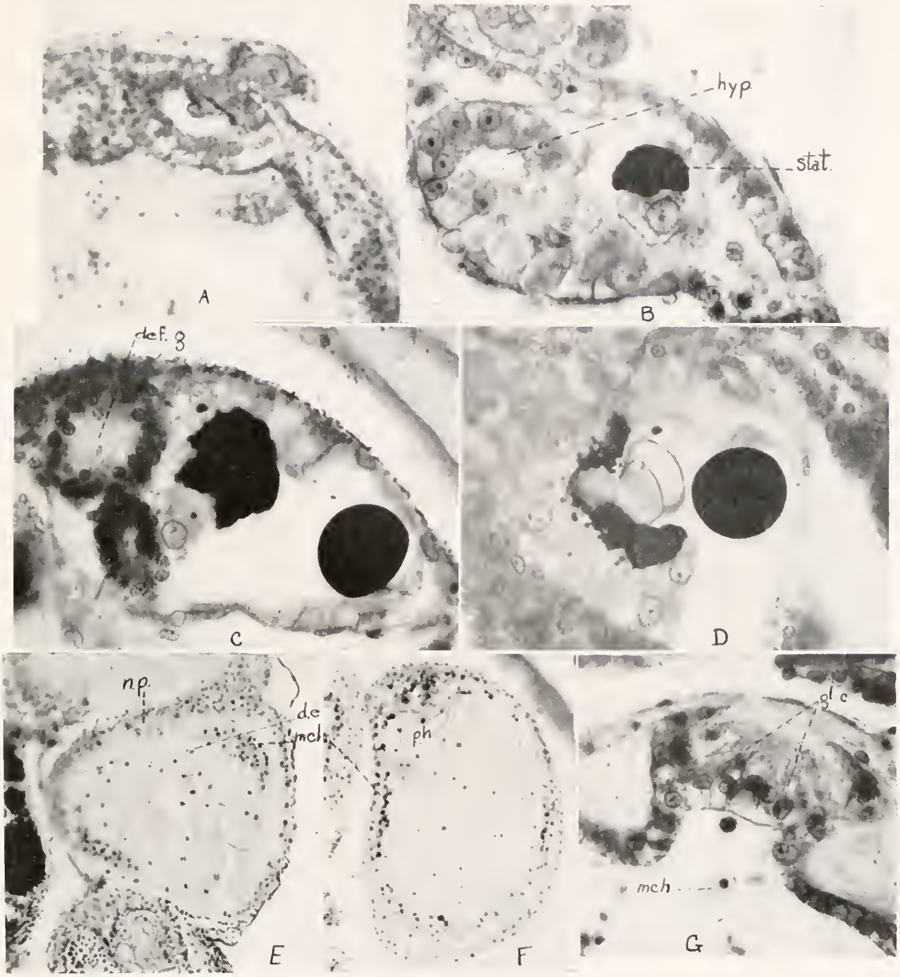

FIGURE 5. *A*. Transverse section corresponding to Figure 4*A*. 225 ×. *B*. Transverse section through brain of Stage III, hypophysis separated from brain vesicle. 650 ×. *C, D*. Sections through sensory vesicle and definitive ganglion of Stage IV; oblique, thus including both sensory organs. 650 ×. *E*. Transverse section through Stage I; anterior region. 150 ×. *F*. Transverse section through early Stage II; neural folds closed. 150 ×. *G*. Longitudinal section through adhesive papilla of Stage III. 650 ×. *d. e.*, definitive endoderm; *def. g.*, definitive ganglion; *gl. c.*, gland cells; *hyp.*, hypophysis; *mch.*, mesenchyme cells; *n. p.*, neural plate; *stat.*, statolith.

Some of the endodermal cells of the yolk mass lie along the right side of the chordal cells and when the tail is constricted from the trunk region these cells form the loose column of caudal endoderm. In Stage IV little of it remains (Fig. 4*C*, 6*B*).

In Stage I the notochordal cells begin to shift in position. They interdigitate into a row of disc-shaped cells occupying the central axis of the short tail. The cells resemble the endodermal cells of the yolk mass in possessing delicate membranes, nuclei smaller than those of adjoining muscle cells, and yolk granules.

During Stages II and III the notochord elongates as the tail lengthens. The chordal cells lengthen; the inter-cell membranes separate from each other converting them into hour-glass shaped cells with the nucleus resting in the constricted neck between the peripheral masses of protoplasm (Fig. 4*E*).

In Stage IV the cell halves separate completely giving the chord the appearance of a tube with a scalloped lining. The proximal end retains its relationship with the hinder end of the pear-shaped mass of yolk between the atrial cavities and the arms of the digestive tract (Fig. 6*C*, *H*). Distally it corresponds in length to the neural tube and tail muscles.

Muscle cells of the tail

Mesoderm differentiates into three structures of the larva, one of which is restricted to the larval action system, two of which function in both the larval and adult action systems. The former includes the muscles of the tail, the latter the muscles of the mantle and mesenchymatous connective tissue in the body cavity. The asymmetry of the posterior lip of the blastopore at the end of gastrulation (Stage I) places the muscle cells of the right side dorsal to the chordal cells and to the right of the neural plate at its posterior end, the muscle cells of the left side to the left of the posterior neural plate but ventral to the notochord (Fig. 4*C*). Each band is made up of four cells in fairly regular rows.

In Stage II the myoblasts are the most prominent cells in the body because of their size and heavy membranes. Each cell contains a large faintly reticular nucleus with a conspicuous nucleolus. The deeper cytoplasm is grossly reticular and retains an occasional yolk granule (Fig. 6*D*).

In Stage III the peripheral cytoplasm elaborates in its cortex, in a slightly spiral direction, along the longitudinal axis rows of contractile fibrillae composed of minute granules so distributed that they resemble the individual myofibrillae of striated muscle of the higher chordates (Fig. 4*E*, 6*D*). The myofibrillae are continuous from one cell to another throughout the length of the muscle bands. Grave (1921) has described this in the free-swimming tadpole of Amaroecium. The bases of the muscle bands, like that of the notochord in Stage IV, are located well within the posterior part of the trunk just behind the mass of yolk (Fig. 6*H*).

Muscles of the mantle

In the late embryonic period (Stage III) many of the mesenchyme cells located directly under the ectoderm unite end to end to form the smooth fibres of the mantle (Fig. 4*F*). One set of such muscle fibres radiates from each of the siphons. The other set encircles the trunk obliquely from the dorsal to the ventral side.

Mesenchyme of the body cavity

In Stage I two compact lateral masses of mesenchyme cells lie pressed tightly between the nutritive endoderm and shallow ectodermal cells. The one on the right side is disposed more dorsally than the one on the left side (Fig. 5*E*). They extend from the posterior muscle cells towards the anterior end of the body.

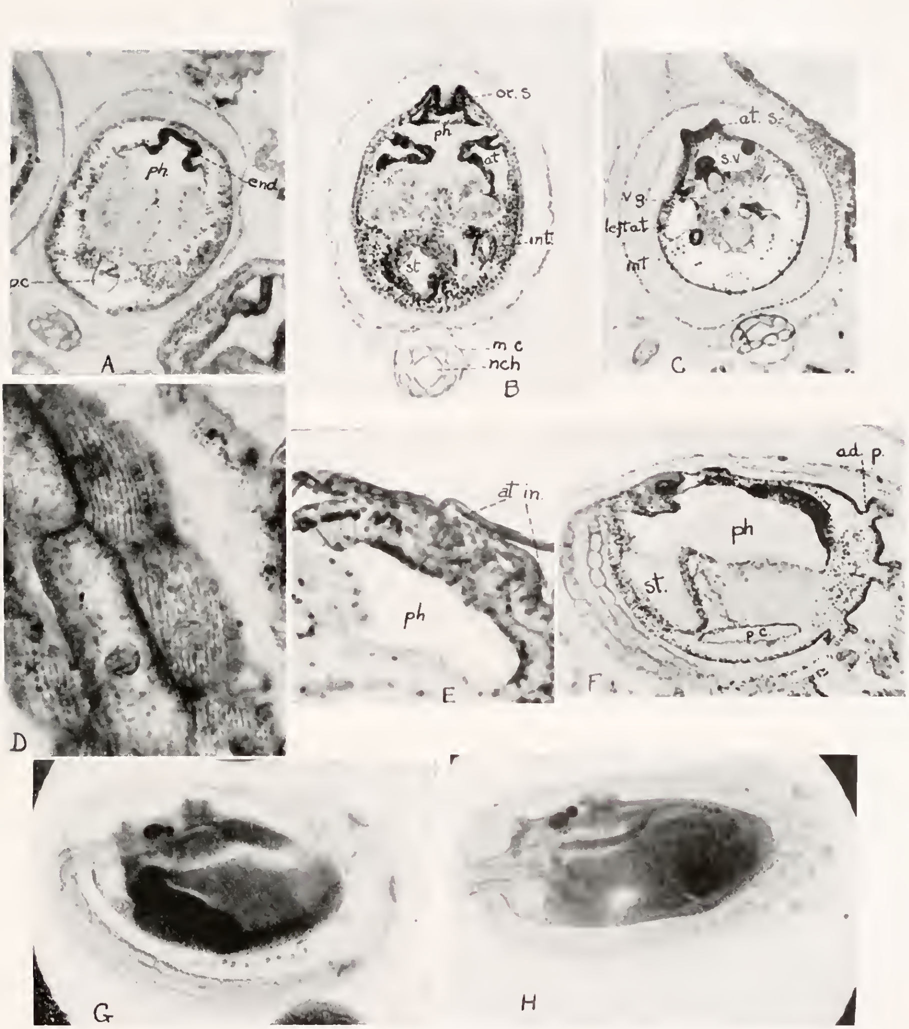

FIGURE 6. *A, B, C.* Transverse sections through tadpoles of Stage IV; *A*, anterior. *B.* In region of oral siphon. *C.* In region of atrial siphon. About 200 ×. *D.* Section through part of muscle band; middle cell through center of muscle cell, lateral cell through peripheral cytoplasm where myofibrillae are formed. About 850 ×. *E.* Transverse section through Stage II to show atrial invaginations. 300 ×. *F.* Longitudinal section through Stage III. About 150 ×. *G.* Tadpole just before hatching, chorion not ruptured. About 250 ×. *H.* Tadpole at hatching. Note insertion of notochord at posterior end of trunk. About 250 ×. *ad. p.*, adhesive papilla; *at.*, atrium; *at. in.*, atrial invagination; *at. s.*, atrial siphon; *end.*, endostyle; *int.*, intestine; *m. c.*, muscle cell; *nch.*, notochord; *or. s.*, oral siphon; *ph.*, pharynx; *p. c.*, pericardial cavity; *st.*, stomach; *s. v.*, sensory vesicle; *v. g.*, visceral ganglion.

In Stage II both masses of cells multiply and spread out under the ectoderm in all directions except posteriorly. A small amount of mesenchyme is found in the tail, probably derived from the cells in the mid-region of the posterior lip of the blastopore. Being crowded together the cells appear angular in section. The nuclei are relatively large (Fig. 4*F*, 5*G*).

During the transition from Stage II to Stage III growth of the body and absorption of the yolk effect a separation between the epithelial cells of the epidermis and the endodermal cells (Fig. 5*F*, 6*F*). As the body cavity enlarges the cells round up, separate from each other, and wander freely about, dividing frequently and eventually filling up all available space except in the area around the base of the tail (Fig. 6*A*, *B*, *C*).

Other mesenchyme cells assume stellate shape and send out long slender strands of protoplasm by means of which they form a reticulum of mesenchymatous tissue. This is the nearest approach to a coelomic epithelium that is found in Tunicates with the possible exception of the perivisceral cavity of Ciona.

The mantle and tunic

The epidermis in Stage I is a layer of thin cells small in surface view dorsally where they adjoin the neural plate, larger towards the ventral body region. In Stage II the cells are of uniformly small size and cuboidal in section except where they invaginate to form the atrium and are columnar in shape. In surface view all present the characteristic polygonal arrangement of epithelial tissue.

During Stage III the protoplasm becomes vacuolated medially, the nuclei being pushed to the periphery where the cytoplasm is more granular (Fig. 5*B*, 6*E*). The epidermal cells grow thinner as development progresses and the inter-cell membranes disappear. When the epidermis has assumed the characteristic appearance of the Tunicate mantle in Stage III it secretes a thick layer of structureless tunic. Occasional cells of the test of the ovum are trapped in the clear tunicin, the greater number, however, being pushed with the test ahead of the tunic (Fig. 6*C*). The tunic is grooved where it is secreted about the tail and when the tail is released, with the disappearance of the test, the groove remains in evidence marking the embryonic position of the tail.

Derivatives of the epidermis

Adhesive papillae—A conspicuous feature of the Amaroecium larva is a vertical row of three adhesive papillae at the anterior end (Fig. 3, 6*F*, *G*, *H*). Each papilla first appears early in Stage III as a local thickening of ectoderm forming a pad of columnar cells. The cells at the periphery of the thickened pad form a stem which increases in length as the tunic thickens, the whole organ becoming goblet-shaped. It retains its connection with the body cavity through its slender hollow stem (Fig. 6*G*, *H*). The papillae extend through the thickness of the tunic and are exposed at its surface. Cells that constitute the functional portion become vacuolated and reticular proximally and toward the center of the cup, where the long cells converge, they produce secretion granules which lodge in the concavity of the papilla (Fig. 5*G*). The bordering epidermis surrounds the disc forming a thin layer over the cup-shaped depression. During the free-swimming life of the larva the secretion granules are converted into a viscid substance by means of which the tadpole becomes attached. The entire glandular structure is of ectodermal origin. Grave (1921) from his study of the fully formed tadpole supposed that mesenchyme cells gave rise to the glandular portion of the papilla. Mesenchyme cells wander from the body cavity into the hollow stalk but they are not incorporated into its structure. The tail encircling the body crowds the papillae a little to the right of the sagittal plane thus adding to the asymmetry of the larva. The three papillae cannot be homologized with the tactile papillae of Botryllus, which are integral parts of the peripheral nervous system. Here they serve only as gross organs of attachment.

Test vesicles—During Stage III, when the adhesive papillae are differentiating, the test vesicles originate as numerous small ectodermal evaginations in four distinct regions at the anterior end of the trunk. Two groups, separated from each other by the median papilla, are directed forward. The dorsal group is derived from a short ridge extending in the direction of the oral siphon. The ventral group, below the ventral papilla, is derived from a long ridge extending posteriorly through about a third of the length of the trunk (Fig. 5, *6G*, *H*). The vesicles themselves originate as independent hollow slender projections of the ectoderm. The attached end of each evagination becomes narrow, finally constricting off and severing its connection at the base. Frequently this separation is not effected by the time of hatching of the vesicle still being attached to the epidermis by their stalk-like bases. When detached the slightly pear-shaped vesicle rounds up and becomes a sphere consisting of a single layer of cells which lose their definition on the proximal side where they are extremely thin.

The use of the word "test" in connection with these vesicles is unfortunate. The chorion of the egg of Tunicates is called the test and the cells that either lie freely in the enclosed liquid or are resolved into pavement epithelium are called the test cells. The tunic of the tadpole is a purely ectodermal derivative. The tunic of the adult colonies being the product of secretory activity of these vesicles, the vesicles should, with greater accuracy, be called the "tunic vesicles."

SUMMARY

1. The digestive system of Amaroecium lacks an open archenteron at the end of gastrulation. The pharynx appears as a narrow incision with a thin roof and heavy floor. An oesophageal evagination differentiates into stomach and intestine.

2. Heart and pericardium originate from the floor of the pharynx.

3. Atrium and siphons are ectodermal structures that become associated with the digestive system.

4. The nervous system consists of a sensory vesicle enclosing two sensory masses of pigment, a hypophysis lying beside two sensory ganglion, a visceral ganglion descending laterally to the neural tube which lies to the left of the notochord throughout the length of the tail.

5. The notocord is derived from chordal cells invaginated at gastrulation. Its cells become vacuolated. The notochord is confined to the tail and posteriormost region of the trunk.

6. Muscle cells differentiate from mesodermal cells of the blastoporal margins. Asymmetry of the blastopore places the cells of its right margin dorsal to the noto-

chord, the cells of the left margin ventral to the notochord. Each band of muscle cells consists of four longitudinal rows. Cells separate from the two lateral masses of mesenchyme and move into the body space of the developing tadpole. They give rise to muscles of the mantle.

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COMPARATIVE SENSITIVITY OF SPERM AND EGGS TO ULTRAVIOLET RADIATIONS

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The sperm of the sea urchin are more sensitive to ultraviolet radiations than the eggs when the effectiveness of the rays is compared by the retardation of cleavage of unexposed eggs fertilized with irradiated sperm on the one hand and of irradiated eggs fertilized with unexposed sperm on the other (Giese, 1939c). It would be interesting to know whether sperm are generally more susceptible to these radiations than eggs; therefore, the experiments were repeated on a number of marine forms. It is also desirable to find an explanation for this differential susceptibility in those cases where it occurs. Insight might be gained by determining action spectra for the sperm and egg, therefore the relative efficiency of action of different wave-lengths of ultraviolet light in retarding cleavage of irradiated eggs and of eggs fertilized with irradiated sperm was determined as described below.

MATERIALS AND METHODS

Arbacia punctulata, Nereis limbata, Chaetopterus pergamentaceus, and *Mactra* sp. were studied at Woods Hole, Mass. *Strongylocentrotus franciscanus* and *S. purpuratus,* collected at Moss Beach, and *Urechis caupo* collected at Bolinas Bay, California, were used at Stanford University, and *Dendraster excentricus* and *Pateria miniata* were studied at the Hopkins Marine Station, each type of egg being used during the active breeding season.

The methods for studying the eggs were similar to those previously described (Giese, 1938). Except for the work on the action spectrum, the mercury argon discharge tube which emits about 85 per cent of its light at λ 2537 Å was used and the light intensity was measured with a Hanovia Ultraviolet Meter (No. 949). The dishes were kept in running sea water to attain a lower temperature than that of the room. The work on the action spectrum was done with a mercury arc and a natural quartz monochromator and the intensity of the light was measured with a thermopile as in previous studies (Giese, 1938). The eggs were kept in dishes in moist chambers and in a constant temperature room at 15° C.

Sperm were used in dilutions of between 1 : 200 and 1 : 1,000 of the spawn. Such dilution is necessary because in denser suspensions ultraviolet light is completely removed by the sperm first reached. Irradiated sperm lose their fertilizing power rapidly, therefore they must be used soon after exposure (see Hinrichs, 1927, for studies on inactivation).

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EXPERIMENTAL

Comparison of various eggs

A summary of the general results obtained with all the eggs studied will be found in Table I. Not all the eggs respond to ultraviolet radiations in the same way. Thus cleavage of eggs of Arbacia and Strongylocentrotus is merely slowed up but remains normal after small and medium dosages so that comparisons of the effects of various dosages and wave-lengths is relatively easy. Abnormalities only appear after larger dosages. In Urechis, Nereis, and some of the other eggs the threshold for abnormal development is relatively low. While per cent of abnormal development could be used for analysis of effects of radiations, it would be much more difficult.

It is readily apparent that with regard to ultraviolet susceptibility, there are two types of sperm: in the Echinoderms, especially Arbacia and Strongylocentrotus, the

TABLE I

Comparative action of ultraviolet radiation[2] *on eggs and sperm of various marine animals*

Species	Effects on eggs	Effects on sperm
Strongylocentrotus purpuratus	Delay just noticeable after about 100 ergs/mm.²; will develop even after 4,000; after 8,000 ergs/mm.² become quite abnormal.	Noticeable delay[3] even after 10–20 ergs/mm.² Marked retardation as dosage above this is used.
Arbacia punctulata	Noticeable delay after 200 ergs/mm.² but even after 2,000 ergs/mm.² plutei, normal but smaller than controls, develop from eggs. After 4,000–8,000 ergs/mm.² eggs are quite abnormal.	Noticeable delay even after less than 50 ergs/mm.² After 250 ergs still develop larvae but after 500 quite abnormal. Even after 4,000 ergs/mm.² sperm activate eggs.
Dendraster excentricus	Slight delay after 1,600 ergs/mm.²; strong after 6,400; quite abnormal after 25,000 ergs/mm.²	Slight delay after 200 ergs/mm.²; abnormal after 800 ergs/mm².
Urechis caupo	Some delay after 200 ergs/mm.² Marked injury with abnormal cleavage after 5,000 ergs/mm.²	Marked abnormalities after 200 ergs/mm.²
Chaetopterus pergamentaceus	Slight delay only after about 4,000 ergs/mm.²; after 16,000 ergs/mm.² still cleave but much delay and many cytolize.	Slight delay after 2,000 ergs/mm.²; killed after about 8,000–16,000 ergs/mm².
Nereis limbata	Even after 4,000 ergs/mm.² develop with little delay to the trochophore stage; after 8,000 ergs show delayed cleavage.	Slight delay between 4,000–8,000 ergs/mm.² 8,000 kills most sperm.
Mactra sp.	Very slight delay after 500 ergs/mm.²; striking after 4,000–8,000.	Delay after 250 ergs/mm.² and progressive delay thereafter.

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[2] The measurements with the Hanovia meter are accurate to about 10 per cent as checked by thermopile measurements in one instance.

[3] Amounting to 15–30 minutes delay at the third cleavage of eggs fertilized with such irradiated sperm. Marked delay means a delay of an hour or more.

sperm are much more sensitive than the eggs; in the worms such as Urechis, Nereis, and Chaetopterus the sperm is slightly if at all more sensitive than the egg, as judged by cleavage delay.

Such a lack of differences in susceptibility of the gametes might be more apparent than real. It is possible that when there is little or no cleavage delay following fertilization of an egg with an irradiated sperm, the sperm may be serving only to activate the egg to haploid parthenogenesis. Eggs of Arbacia and of Chaetopterus were, therefore, fertilized with sperm treated either to a small dosage or to a medium dosage of radiations and at appropriate intervals samples were fixed in Bouin's fluid and stained with iron hematoxylin. Although the preparations were not entirely satisfactory, evidence for pronuclear fusion was observed in both cases. No lagging or disintegrating sperm were observed in the cytoplasm of either egg. Since neither cytological nor physiological evidence suggests parthenogenesis, it seems likely that for the dosage ranges tested the delayed cleavage follows fusion of the gametic nuclei. The difference between the two types of sperm must lie in some other factor. Possible explanations will be considered in the discussion.

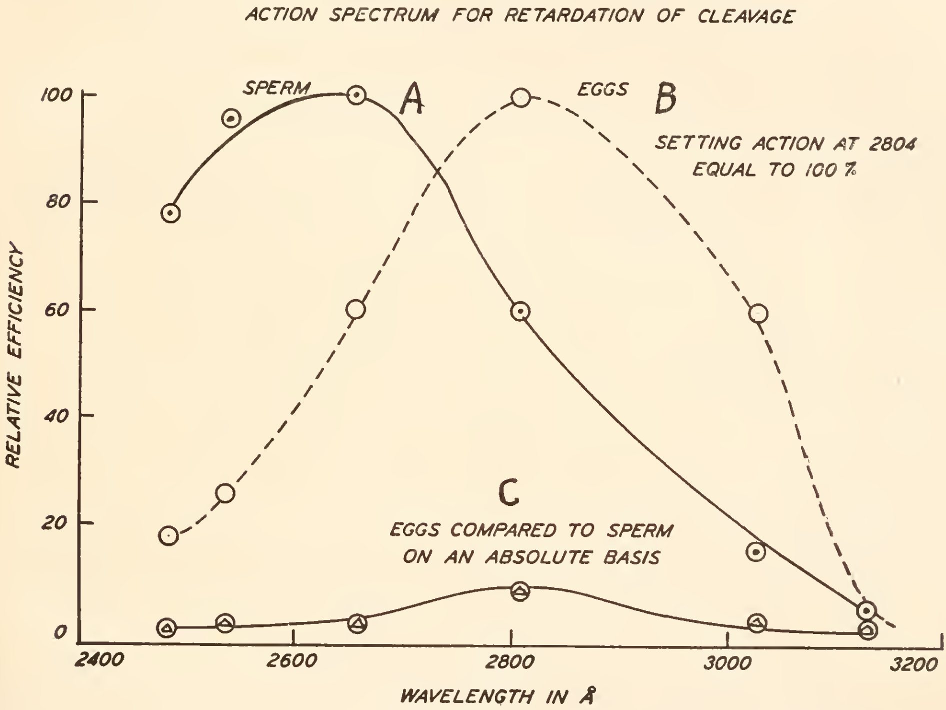

FIGURE 1. Action spectra for retardation of cleavage of eggs fertilized with irradiated sperm at *A* and for irradiated eggs at *C*. At *B* the data for the eggs are compared on a relative basis setting the value at λ 2,804 Å as 100 per cent efficient. See text below.

The data in Table I show that the threshold for effects on cleavage is quite different for eggs of different species. Thus Strongylocentrotus, Arbacia, Mactra, and Urechis eggs are retarded after brief exposures to ultraviolet as compared to

Nereis, Chaetopterus, and Dendraster. Whether this is due to mere physical screening by some inert materials in the egg or to differences in concentration of some light sensitive materials is not known.

Action spectra for egg and sperm

If irradiation of the nucleus alone causes retardation of division of the cell, the same action spectrum should be found for egg and sperm; that is, there should be no qualitative difference in effectiveness of different wave-lengths even though the general susceptibility of the sperm is greater. If elements in the cell other than the nucleus are involved the egg may show an action spectrum different from that of the sperm.

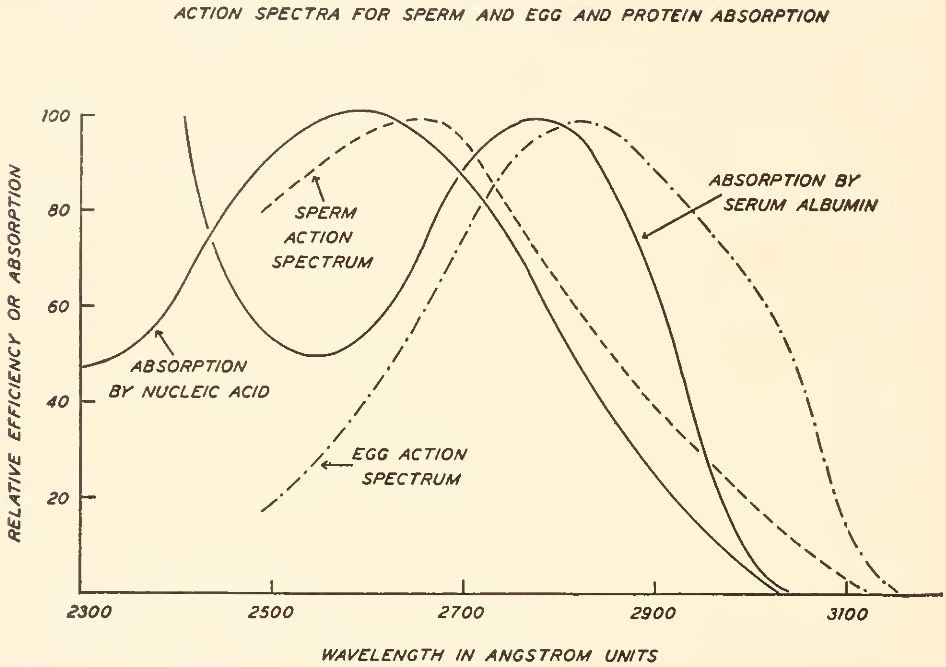

Figure 2. Comparison of the action spectra of Figure 1 with absorption spectra of nucleic acid and serum albumin. Data for nuclei acid from Caspersson (1938), for proteins from Smith (1929). Note that the action spectrum for the egg is quite different from the absorption spectrum for albumin at both ends.

The methods employed for the studies at different wave-lengths are similar to those already described elsewhere (Giese, 1938, 1939c), therefore, only the briefest mention need be made of them. The irradiated eggs are fertilized with normal sperm. The rate of division is then determined by observing for percentage of cleavage every 15 minutes. The times at which the eggs reach the 2, 4, 8, 16, and 32 cell stages are recorded and the number of cleavages is plotted against the time after fertilization and compared with the control. The increase in time required to reach the third cleavage is taken as a measure of the retarding action of the radiations. The retardation is then plotted against dosage. From such curves for each of the wave-lengths the dosage required to bring about a given retardation can be determined. For Figures 1 and 2 the reciprocals of the relative amounts of energy at different wave-lengths required to produce a retardation of division by 1.5 hours were determined. In Figure 1 at *A* and *C* the sperm and egg are compared on this basis and a great difference in susceptibility between the gametes is evident. In *B* the data for the eggs are compared amongst themselves on a relative basis setting the action at λ 2,804 Å as 100 per cent efficient.

The shape of the curves indicates that different materials are being affected in the two cases, since the action spectrum is considered to be a measure of the absorption by the active constituent. To see if the absorbing materials can be identified the absorption spectra for serum albumin and nucleic acid are given in Figure 2. It is apparent that the action spectrum for sperm matches the absorption spectrum for nucleic acid better than the absorption spectrum for albumin; the reverse is true for the action spectrum of the egg. Since the simple proteins and nucleoproteins are the major structural constituents of the cell and none of the other organic or inorganic constituents have very specific absorption, the resemblances while imperfect are indicative of absorption by these two classes of compounds in the action of ultraviolet radiations on the gametes.

Discussion

The occurrence of a differential susceptibility of gametes with the sperm more sensitive to ultraviolet light than the egg as first found in the sea urchin, *Strongylocentrotus purpuratus,* was verified on Arbacia and Dendraster and in preliminary trials on Pateria and *S. franciscanus* but not on Urechis, Chaetopterus, and Nereis. In the latter forms the sperm appears to be only slightly more sensitive than the egg (Table I). The former group of species belongs embryologically to the radially-cleaving, indeterminate egg type, the latter group to the spiral determinate type. In addition, the radial eggs used here are mature or nearly so at the time of shedding whereas the spiral eggs are generally immature. An illustration of the difference in response to ultraviolet light, depending on this difference in organization is seen in the local "burns" occurring in the spiral eggs. Thus a Nereis egg given a unilateral dosage of between 8,000–16,000 ergs/mm.^{2} may develop apparently normally except on the burned surface which appears blistered. A Strongylocentrotus egg on the other hand unless given a large dosage of light will show general effects distributed throughout the retarded egg. However, it is not possible to say which features of the organization account for the difference in sensitivity of the eggs and sperm of the two groups.

One might envisage that in eggs the retarding effects of radiation on cleavage are due to the inactivation, by substances formed during irradiation, of some catalyst which is necessary for the reactions involved in cleavage. In one group of eggs perhaps the catalyst is present in excess of that necessary for a characteristic rate of cleavage, the rate being controlled by some other limiting factor, in the other it is present in just adequate concentration and itself constitutes the limiting factor. Even a considerable dosage of radiations will not reduce the concentration of catalyst below the critical level in the first case but will readily do so in the second. In the first case no cleavage delay would be expected until very large dosages of radiations had been administered, in the second the cleavage should be affected after very small dosages. One would have to assume that irradiated sperm on penetrating unirradiated eggs introduce similar cleavage-inhibiting substances acting on the catalyst as those formed in the irradiated egg. In this case also the effect on cleavage should depend upon the amount of catalyst present in the egg—if in excess, the cleavage should not be easily inhibited, if limiting, the reverse should be true. We should expect both sperm and egg to be relatively insensitive to the radiations in the former and this is found in most spirally cleaving eggs.

Against the above postulation is the fact that the action spectrum for sperm resembles nucleoprotein absorption while for the egg it resembles simple protein absorption indicating two different ultraviolet absorbing materials in the gametes by which the cleavage-retarding effect is produced. It is possible that absorption by both of these types of proteins leads to the formation of toxic photoproducts which inhibit the same catalyst. It is also possible that the toxic substance is much more rapidly formed by the nucleoproteins, but the necessary assumptions strain the imagination.

It should be pointed out that the retardation of the early cleavage is only the initial effect of the radiation. If the delayed effect could be studied we might find that the recovery from injury to the egg would resemble absorption by nucleoprotein, indicating a more lasting injury to the nucleus than to the cytoplasm, as is the case for division of Paramecium (Giese, 1945a). Because the number of cells cannot be satisfactorily determined in the later cleavages such experiments were not attempted with eggs.

The action spectrum obtained for the egg is similar to that observed for "cytoplasmic" effects such as increased time of ciliary reversal, retardation of excystment, immobilization of cilia, and prevention of hatching of eggs. The action spectrum for the sperm resembles that for "nuclear" effects such as recovery of paramecia from sublethal effects, bactericidal and fungicidal effects and the production of mutations (see Giese, 1945b, for references). It is interesting to note the difference between the action spectrum for retardation of cleavage of the egg and for activation studied by Hollaender (1938). In the latter case no action was found until about λ 2,650 Å and the effectiveness of the light increased as the wave-length decreased. The mechanism of action of the light must be different in these two instances. The action spectrum data thus lay the foundation for further analysis of the effect of these radiations upon gametes.

SUMMARY

1. The action spectrum for the retardation of division of eggs fertilized with irradiated sperm resembles the absorption of ultraviolet light by nucleoproteins.

2. The action spectrum for retardation of division of irradiated eggs of the sea urchin resembles absorption by simple proteins like albumin except that at the short wave-length end there is no increase in action at λ 2,483 Å where absorption shows a definite upswing.

3. The absolute amount of energy required to affect division to the same extent by affecting the sperm is very much less than that required to affect eggs.

4. Other Echinoderms tested show a similar difference in susceptibility of eggs and sperm: *S. franciscanus, Arbacia punctulata, Dendraster excentricus,* and *Pateria miniata*

5. Animals other than Echinoderms tested did not show as striking a difference between susceptibility of eggs and sperm: *Urechis caupo*, *Mactra* sp., *Chaetopterus pergamentaceus*, and *Nereis limbata*.

6. In the eggs listed in paragraph 5, determinations are made more difficult by the tendency for the eggs to show irregular cleavage rather than retarded cleavage as the dosage increases. Such irregular cleavage occurs in Echinoderm eggs as well but the threshold is higher.

7. If both eggs and sperm of the sea urchin are irradiated the effect on the rate of division is less than the sum of the effects which would be expected on each of the gametes alone. However, the percentage of abnormal cleavage greatly increases.

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OBSERVATIONS ON THE FUNCTIONING OF THE ALIMENTARY SYSTEM OF THE SNAIL LYMNAEA STAGNALIS APPRESSA SAY

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INTRODUCTION

Although records exist of functional studies on the alimentary system of Basommatophora as far back as the early eighteen hundreds, the detailed story of the course and ultimate fate of food in the alimentary tract and the simultaneous movements of the tract is thinly scattered and far from complete. In the more recent emphasis placed on some gastropods because of their importance as vectors of parasites of man, domestic animals, wild game, and fish, it is vitally important that the normal physiology of the system most frequented by these parasites be better known.

It is the purpose of this paper to integrate the previous work on the physiology of the alimentary system of *Lymnaea stagnalis* and allied forms (suborder Basommatophora, order Pulmonata) with original research on the same system in *L. s. appressa* Say. The basic morphological (Carriker, 1945) and histological (Carriker and Bilstad, 1946) studies on this system in *L. s. appressa* have been completed and are in press. All terms used in this research have been described in these two papers.

L. s. appressa has been selected for this research because it is a representative vector and because of its excellent response to laboratory culture, its relatively large size (maximum shell length, 62.5 mm.) as compared with other fresh water pulmonates, its short life cycle, and its relatively thin semitransparent shell and semitransparent tissues. Snails used in the research were cultured entirely in the laboratory. They were grown through many generations in large battery jars and fed on lettuce and cooked "cream of wheat" cereal. The water in the jars was aerated by means of a small Marco air pump (Noland and Carriker, 1946). The original snails were collected in Fox Lake, Wisconsin, in 1939. Parasite-free cultures (especially of trematodes) from the original snails were obtained by the isolation of the egg mass soon after oviposition in separate aquaria. Each new culture was started in this way rendering transmission of infection very improbable. Detailed examination of succeeding generations has not disclosed parasites.

This work was carried out at the University of Wisconsin (1939–1943) under the stimulating guidance of Prof. L. E. Noland, whose advice, encouragement, and friendly cooperation were much appreciated.

HISTORICAL REVIEW

Scanty observations on the function of the anterior part of the alimentary tract of *Lymnaea* were given by Semper (1857), Geddes (1879) and Moquin-Tandon (1885); more detailed information was given by Amaudrut (1898), Pieron (1908) and by Baecker (1932). The stomach region was investigated by Gartenauer (1875), Moquin-Tandon (1885), Colton (1908) and Heidermanns (1924). These experimental contributions of Colton and of Heidermanns, particularly of the latter, are noteworthy. The liver has been the object of most of the physiological work although the research has usually been incidental to that on the stylommatophoran *Helix:* Barfurth (1880, 1881, 1883a and b), Frenzel (1886), Cuénot (1892), Enriques (1901, 1902), Faust (1920), Peczenik (1925) and Krijgsman (1928). Only the investigation of Peczenik is exclusively on *L. stagnalis.*

Experimental Methods and Results

Lymnaea physiological salt solution

The study of the living system has required the development of a physiological salt solution which will approximate the ionic and osmotic balances of the blood of *Lymnaea* more closely than do such commonly used solutions as Ringer's. On the basis of incomplete data given by Duval (1928) on *Lymnaea* and by Bernard and Bonnet (1930) on *Helix* on the molecular concentration of blood, the following solution was developed for *L. s. appressa:*

$NaCl$	2.0 gms. per liter
$NaHCO_3$	2.0 " " "
KH_2PO_4	0.1 " " "
$MgCl_2$	0.3 " " "
$CaCl_2$	0.3 " " "

This solution consists of 0.47 per cent salts and gives a pH of approximately 7.8. After about a week considerable precipitation of $CaCO_3$ occurs, although this seems to have no noticeable effect on the isolated organs. The vas deferens was used in testing the solution and was found superior to the heart for this purpose. The vas deferens, terminal preputium and prostate gland were removed under the physiological salt solution from the cephalic hemocoel without bruising. This portion of the reproductive tract is in part a strong muscular tube which is easily excised and maintains a continuous squirming motion as long as the tissues are alive. It continued squirming for about 66 hours in the solution described above. A Ringer's solution of 0.7 per cent salts keeps it moving for about 12 hours, although at a much reduced rate.

Hydrogen ion concentration

The first work on the estimation of the pH of the alimentary tract of a fresh water snail seems to be that done by A. H. Rosenbloom on *L. s. appressa* in his bachelor's thesis in 1942 (unpublished) in this laboratory. He has kindly consented to the incorporation of his results in this paper. His method was essentially the colorimetric one employed by Yonge (1925): fluids from the various lumina of the alimentary tract of the snails under variable feeding conditions were pressed out onto paraffined plates and thoroughly mixed with indicators (brom-thymol blue, neutral red, and methyl red). The colors were compared with those of indicators freshly prepared in buffered solutions checked on a Coleman pH electrometer. The results are given in Table I:

TABLE I

pH of the contents of various lumina of the alimentary tract of L. s. appressa

Organ	Average pH	Maximum and minimum pH	Number of tests	Number of snails
Buccal cavity	Same as water in external medium.			
Proesophagus	6.9	7.2–6.3	10	10
Postesophagus	7.2	7.6–6.6	10	10
Gizzard and crop	6.4	7.2–6.3	12	12
Pylorus	6.6	7.0–5.8	10	10
Intestine	7.1	7.8–6.2	35	15

Enzymes

Preliminary tests were made for non-purified cathepsin, pepsin, trypsin, and amylase. The tests for the proteinases were made according to the methods of Anson (1938), Bradley (1938) and Folin and Ciocalteu (1927); those on amylase, by the iodine test of Hawk and Bergeim (1937). Semi-micro technics were applied to large numbers of the excised organs.

Maximum catheptic activity (at pH 3) over a ten-day period was found in the liver. That occurring in the buccal mass and gizzard and other portions of the alimentary system was not significant as compared to that in the liver. In an effort to determine to what extent cathepsin might be secreted from the liver, gut fluid from which the amebocytes had been centrifuged was tested. Under the conditions of the experiment, at least, no cathepsin was found in the gut juice. In some tests tryptic activity was found in the salivary glands. A very active amylase, optimum pH 7, was present in the salivary glands and in the liver.

The only investigation of the hydrolytic enzymes of the alimentary system of the Basommatophora reported in the literature is that by Heidermanns (1924). He described a positive test for cellulase present in the digestive juice of the stomach organs (crop, gizzard, and pylorus) of *L. stagnalis.*

Ciliation

Ciliary currents were studied by the injection of fine carmine suspensions in *Lymnaea* physiological salt solution through various portions of the exposed tract, by application of carmine particles to the epithelium of the opened tract and by placing small bits of gut wall in a carmine suspension on an uncovered microscopic slide under high magnification. In some dissections the undisturbed food particles were seen passing through various portions of the excised gut on the natural ciliary currents.

No work has been performed previously on the ciliation of the alimentary system of the Basommatophora. Merton (1923) in his research on the external ciliation of pulmonates included a brief study of the ciliation of the hepatic ducts of *Helix.*

The entire alimentary system of *L. s. appressa,* with the exception of the gizzard and portions of the buccal cavity, is ciliated (see later in this paper), Figures 3, 9, and 11.

Muscular activity

The activity of the alimentary system was observed under binoculars through the transparent walls of normal living young snails and in adult unanesthetized snails opened under *Lymnaea* physiological salt solution. The independent activity of the radula over the odontophore was clearly observed and conclusively verified by watching snails under the binocular under the following conditions: snails deprived of food for a day were placed in a finger bowl of well aerated water to which had been added strips of lettuce (1–2 mm. wide). A Petri dish was floated over the lettuce and the water. As the snails crawled upside down under the glass, feeding on the lettuce, the action of the radula and mouth parts was clearly visible under a strong beam of light.

Sand in the gizzard

In order to check the experiments of Heidermanns (1924) and to add additional information on the role of sand in the comminution of food by the gizzard of *L. s. appressa,* the following experiments were devised.

Sixteen adult snails were placed in each of four aerated aquaria containing a one-half inch mesh wire platform over the bottom. By means of this contrivance the feces were removed from the vicinity of the snails soon after defecation. To three of the aquaria the following foods were added respectively: (1) cooked "cream of wheat," (2) filter paper, and (3) lettuce. (4) No food was added to the fourth tank. (5) A fifth tank was assembled as a control without the wire platform and with lettuce and sand. One snail from each aquarium was killed daily and opened immediately. After ten days the following was disclosed: eight of the forty-three experimental pulmonates contained no sand in the tract, thereby showing that it is possible to rid completely the tracts of a few of the snails of sand; however, there was extensive variation in the ability of the different snails to retain sand. As the quantity of sand in the gut decreased, the snails consumed less food, until in the absence of sand in the tract, no food was ingested and the guts became void of food material and feces. The different diets indicated no significant difference in their respective values as sand eliminators. Sand was found most abundantly in the gizzard lumen, then in decreasing amounts in the crop and retrocurrent passage of the pylorus (anatomical terminology has been described elsewhere, Carriker, 1945). After the quantity of sand in the lumen of the gizzard reached a certain low level, it was retained with surprising tenacity for many days. The material in the fecal pellets of the control snails, particularly of the gizzard residues, was markedly brown and more thoroughly triturated than those of snails with sand-free diets.

In a second set of experiments snails approximately 10 mm. in length were placed in a one-quarter inch mesh wire basket suspended in a large laboratory snail stock tank. The feces, propelled by the sluggish circulation of the water in the tank, passed out of the basket. All lettuce placed in the basket was carefully washed to remove sand. The experiment was continued for several months. In spite of precautions, small quantities of fine sand were always present in the tracts of some of the animals; however, this did not seem to be enough for proper trituration as many of the snails died abnormally at an early age and none reached the normal adult size of the control snails in the tank outside the experimental basket. There is unquestionably a vital need for the presence of at least a limited quantity of sand in the gizzard of these snails for sufficient breaking down of the food.

These results are in agreement with the findings of Heidermanns (1924) and of Colton (1908). Heidermanns accidently discovered that the only way to entirely remove the sand from a live snail was to cause it to hibernate, in which state it emitted the total contents of the tract. Colton noted that in the presence of sand the plant food was cut to pieces by *L. columella*, but that in the absence of sand it went unmolested.

Digestive cell ingestion

By the use of a method patterned after that of Peczenik (1925) the ingestion of particulate food by the digestive cells was investigated. White of egg was strained through cheese cloth. Carbon (lamp black) was ground into the egg albumen and the mixture was thoroughly beaten. This was steamed to a stiff mass and fed to snails starved for a few days. After feeding commenced, the snails were opened every other day. Indigestible residues within vacuoles in the digestive cells as well as similar residues in the fecal pellets showed the presence of very minute particles of carbon, particles not present in the control snails. The indigestible residues in the digestive cells appeared very similar to the albumen passing down the intestine in the gizzard residues.

Fecal rhythms

Some information was gathered on the rhythms of the liver and of the gizzard by a study of the rate and extent of passage of the various fecal strings. The fecal pellets of a 40 mm. snail were observed daily for twenty-four days. The animal was isolated in a two-liter glass jar over the bottom of which was placed a paraffined one-half inch mesh galvanized metal screen, so that all fecal pellets fell to the bottom of the jar and could not be reconsumed. The mollusc was fed lettuce on which was sufficient sand for the needs of the stomach region. Three egg masses were oviposited by the snail, and it added 2 mm. of shell during the twenty-four day period. Upon dissection at the end of the experiment the animal appeared normal in all respects. For the first ten days the pellets were collected and examined microscopically every few hours during the day; during the latter part of the experiment they were collected every twelve hours. Numerous examinations were made of fecal pellets from the stock snail tanks to corroborate the findings on the experimental snail.

Physiology of the Alimentary Tract

Buccal mass and esophagus

L. s. appressa is primarily an herbivore. In the laboratory it may complete its life cycle on lettuce alone and in its natural state feeds on the aquatic vegetation of its surroundings. Specialization of the alimentary system (Carriker, 1945) has been in keeping with a plant diet. However, animal food is also consumed as has been observed by Walter (1906) and by seven other authors cited by him. Repeatedly in this laboratory *L. s. appressa* has been observed to eat the bodies out of the shells of dead snails in the aquaria. Biochemical tests disclose the presence of some tryptic activity in the secretion of the salivary glands.

Pieron (1908) has found in *L. auricularia* and *L. stagnalis* that there is a total absence of food discrimination in the buccal mass and that their feeding is a reflex which keeps the radula working most of the time. The only portion of the body showing any discrimination is the anterior surface of the foot which contains faintly sensitive chemoreceptors. In aquaria in this laboratory *L. s. appressa* rasps much of the time, whether on lettuce or over the newly cleaned glass surface of its tank. However it does also pass through regular "resting" periods in which no rasping occurs. In the rasping stroke the radula passes first to one side and then to the other describing a broad feeding track.

Feeding can be followed clearly in normal immature "albino" *L. s. appressa* (a strain with very little dark pigment) feeding on a "cream of wheat" food mixture blackened with lamp black. This can be seen to pass as far as the stomach region. On the protractor stroke the radula cups to an elongated spoon-shaped trowel about one-half the width of the upper mandible, and working against this, cuts out long narrow bits of food. Each denticle is sharp so the concerted action of the numerous denticles on the radula, sliding independently over the odontophore, provides an effective cutting-rasping apparatus. The food bits are pushed back through the dorsal food channel to the rear of the buccal cavity which dilates to receive them. The tip of the radula closely appresses to the dorsal wall of the buccal cavity in its rearward passage, as attested by the jagged pattern of the dorsal chitinous surface. The buccal aperture constricts strongly and rapidly after the receding radula. Some bits of food are dropped and remain in the dorsal food channel for the next rearward swing of the food-laden radula. Several food bits clump in the rear of the buccal cavity prior to being forced down the esophagus. The radula functions principally in cutting pieces of food of suitably small dimensions for convenient transport through the anterior portion of the alimentary tract; it does not triturate the food to any considerable degree.

Only the posterior third of the buccal cavity is ciliated. These cilia and those in the densely ciliated esophagus beat strongly posteriorly, bearing food bits from the rear of the buccal cavity to the crop.

In connection with the functioning of the buccal mass, refer to a previous paper (Carriker, 1945) for the names, origin, and insertion and relations of the muscles and parts of the mass. The muscular activity of the buccal mass is divisible into four major synchronous movements: (1) opening and closing of the oral aperture and consequent spreading and approximation of the mandibles and lips, as well as dilation and contraction of the circular muscles about the anterior portion of the buccal cavity, (2) backward-forward and simultaneous elevator-depressor movements of the odontophore, with some slight turning of the odontophore on its longitudinal axis and some movement to the right and to the left, (3) movement of the radula and radular sac over the cartilage, and (4) backward-forward and simultaneous elevator-depressor movements of the entire buccal mass. Consequently there exist in the buccal mass three intrinsic focal points about which the majority of the muscles radiate: (1) the oral aperture, (2) the odontophoral cartilage, and (3) the radula and the radular sac.

The activity of the odontophore with respect to the remainder of the buccal mass may be arbitrarily divided into four phases, and described as follows: (1) the *quies-*

cent stage in which the odontophore lies at rest in the rear of the buccal cavity with its longitudinal axis in a dorsoventral position. (2) The *protracting stroke* in which the proximal end of the odontophore swings in an arc of about 130° from its basal position to a point where it lies above the plane of the distal end, which then is in a position to pass partly out of the buccal cavity, bringing the radula against the substratum. At the beginning of this stroke the odontophore assumes a horizontal position as a result of the lowering of the distal end by contraction of the dorsal odontophoral flexor muscle, and a simultaneous raising of the proximal end by strong contraction of the posterior jugalis muscle. The oral aperture and the anterior portions of the buccal mass dilate to permit partial protrusion of the odontophore through the mouth; the labial retractors, suboral dilators and dorsomandibular dilators spread the mouth. The extrinsic postventral levators and posterior jugalis further raise the rear of the buccal mass so that the distal tip of the odontophore is directed towards the oral aperture, to which it seems to be guided principally by the action of the dorsal odontophoral flexor muscles. The inframedian radular tensors draw the radula over the distal end of the cartilage to the point where most of the radula outside the radular sac lies on the under side of the horizontally inclined cartilage, and the collostylar hood lies just behind the distal crest of the cartilage. The combined action of the radular sac and cartilage tensors holds the radula tautly drawn over the cartilage in readiness for the rasping stroke. Contraction of the intracartilage tensors adds considerably to the rigidity of the cushion under the radula. As Woodward (1895) points out for *Natalina caffra,* the fibers of the cartilage act in much the same way as the intrinsic muscles of the human tongue and in contraction cause an elongation and consequent slight protrusion of the radula. The pressure of the blood in the odontophoral sinus probably provides further turgidity. Contraction of the extrinsic as well as of the intrinsic protractor muscles brings the odontophore to the substratum. (3) In the *rasping stroke* the distal tip of the odontophore is drawn over the substrate in a licking motion. The radula, independent of the principal motion of the cartilage under it, is itself simultaneously slid quickly backward most of its length over the cartilage by the action of the heavy supralateral and supramedian radular tensor muscles. The odontophore is aided by contraction of the extrinsic preventral levator muscles which pull the anteroventral floor of the buccal cavity forward and upward. As the mouth opens during the previous stroke, the cutting distal margin of the dorsal mandible is turned partly forward by contraction of the dorsomandibular dilators and possibly the posterior jugals. Thus as the radula rasps forward it makes connection with and scrapes past the inner side of the dorsal mandible, much as two jaws would come together, so that the snail when feeding on thin portions of lettuce actually "bites" off pieces with each rasping stroke. It is only when feeding on thicker foods that true "rasping" comes into play. The dorsal mandible is governed by the dorsomandibular approximator muscle. The lateral mandibles afford mechanical protection to the sides of the mouth, and close in medially after the radula and under and behind the dorsal mandible. (4) The *retractor stroke* returns the odontophore to the resting condition, and completes the cycle, by action of the extrinsic retractor muscles and the supralateral and supramedian radular tensors and relaxation of the protractors. The oral aperture is closed after the receding odontophore by action of the labial sphincter and the mandibular approximator muscles; the buccal cavity, by a contraction of the buccal sphincter and related muscles of the walls. In assuming the resting position, the radular sac is depressed behind the cartilage and the radula rests principally behind the vertically arranged cartilage so that the ventral tip of the sac projects slightly below the level of the buccal mass. As observed by Amaudrut (1898) for *Lymnaea*, the ventral wall of the buccal cavity between the esophageal ledge and the collostylar hood is also depressed, forming a slight dilation in front of the esophageal opening. As both the oral aperture and the proesophagus are closed during the retractive stroke of the radula, it is likely that this dilation is instrumental in creating a slight vacuum in front of the esophageal opening which aids in disengaging food particles from the radula. The dilation is caused principally by depression of the radular sac and possibly by contraction of the superior suspensor muscle of the radular sac and the hood tensor muscles.

The proesophagus is limited in its muscular activity to slight peristaltic waves proceeding towards the postesophagus; while the latter undergoes pronounced peristaltic activity in either a forward or a backward direction, dilating broadly and contracting its entire length. In dilation it may become so large as to fill much of the cephalic hemocoel of the expanded mollusc. In expansion it is filled with a reddish fluid from the stomach region and food particles.

In the buccal cavity the food receives generous quantities of fluid from the buccal gland cells, a fluid which is probably mostly mucoid in nature, judging from the positive mucicarmine stain and from negative tests for amylase and trypsin. This does not however preclude the possibility of the presence of other enzymes which were not tested for. As food passes under the openings of the salivary ducts it receives mucus, amylase, trypsin, and possibly other enzymes from the salivary glands.

The proesophagus adds more secretion from buccal glands and mucous cells. The postesophagus functions as a temporary reservoir for the retention of food when the crop is full. Being capable of considerable distension, it may retain larger quantities of food than the crop. Digestion begins in the postesophagus because of enzymatic secretions received from the salivary glands.

Stomach region

Comminution of food particles is completed in the crop, gizzard, and anterior portions of the retrocurrent passage of the pylorus. These three organs act as a unit comparable to a grist-mill. The kneading motion of the anterior and posterior gizzard constrictor muscles and the gizzard lobes over the sand in the lumen provides the grinding action. Food bits forced between the sand are soon crushed to minute particles upon which the digestive enzymes may act more efficiently. Two synchronized movements are present in the gizzard. In the first the anterior and posterior gizzard constrictor muscles alternate smoothly in mild contraction, thus mixing and forcing the contents of the gizzard slowly back and forth; in the second, not as frequent as the first, the bulk of the gizzard compressor muscles contract suddenly and strongly, bringing pressure to bear on the contents of the gizzard. The presence of gritty material in the gizzard of the Lymnaeidae has been noted by many: Cuvier (1817), Wetherby (1879), Whitfield (1882), Moquin-Tandon (1885), Colton (1908), F. C. Baker (1900, 1911), and Heidermanns (1924).

In the crop, all ciliary currents lead to the anterior margin of the right gizzard pad, those on the left side beating ventrad and over to the right (Fig. 3). Thus fine food material accumulates on the right side of the crop at the anterior edge of the right gizzard lobe. The crop receives food from the postesophagus and forces it into the gizzard lumen. When ample sand is accessible to the animal, the crop and anterior portions of the retrocurrent pyloric passage are both filled with it. The walls of these organs act as mechanical obstructions to the open ends of the gizzard lumen and concentrate the pressure of the gizzard musculature upon the contents. They also cooperate in the muscular activity of the gizzard in a unified kneading and a slow rotation of the gritty contents. The retrocurrent passage returns to the crop those particles which have been dislodged from the gizzard contents by muscular movements of the stomach region. In this fashion the contents of the gizzard undergo thorough comminution and partial digestion before the residues are shunted down the procurrent passage to the prointestine.

The epithelium of the stomach region bears a complicated system of ciliary currents (Figs. 1, 2, 3, 9). Cilia in the procurrent passage direct fine particles from the right ventral side of the gizzard cavity to the prointestine. Those in the retrocurrent passage are directed anteriad towards the left side of the gizzard cavity. The dorsal passage bears what in fixed sections appears to be nothing more than a brush border. Even in carmine suspensions under high magnification no distinct current could be detected in it. The cilia on the ventral fold are divided into two distinct functional areas: those on the right half of the fold beat obliquely posteriad and laterally in the direction of the currents in the procurrent passage; those on the left half, obliquely anterolaterad in the direction of the gizzard and the currents in the retrocurrent passage. The currents on the minor fold whip obliquely anterolaterad; those on the medial half of the major fold pass obliquely anterolaterad; while those on the lateral half of the major fold and those on the medial half of the fold adjacent the hepatic vestibule reach posterolaterad. The ciliary currents in the retrocurrent passage are noticeably faster than those in the procurrent passage. Currents on the atrial corrugations run into the incurrent tubule of the cecum. Thus the pylorus in cross section (Fig. 2) is composed of three channels, each with distinct ciliary currents and of three folds which almost meet centrally and whose

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EXPLANATION OF PLATE I

(All figures concern *L. s. appressa*)

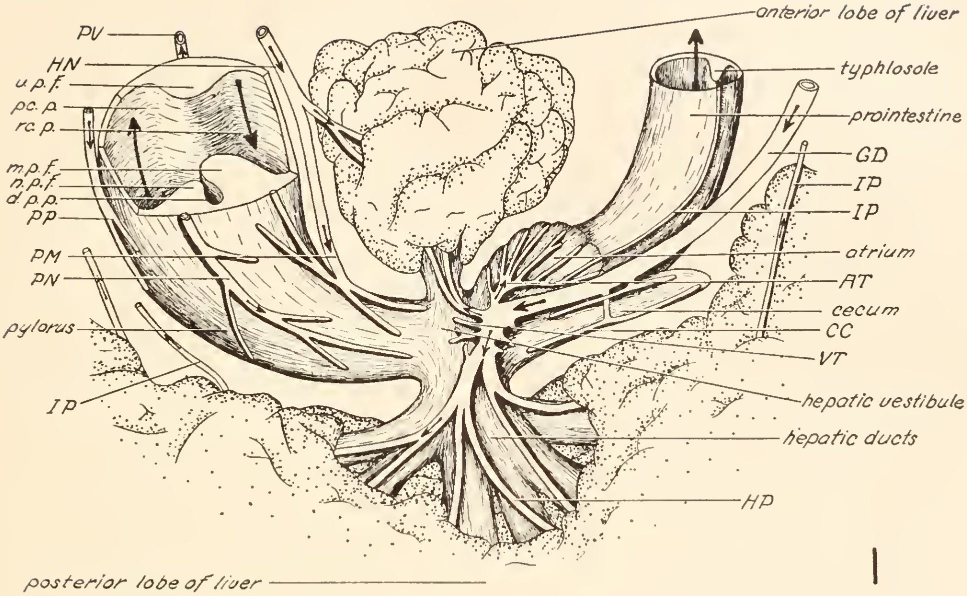
FIGURE 1. Stereogram of the pylorus, hepatic vestibule, atrium, cecum, anterior portion of prointestine, and liver lobes. The vascularization is stressed. (Small arrows indicate the flow of blood in the arteries; large arrows, the direction of movement of the contents of this part of the tract.) × 6.

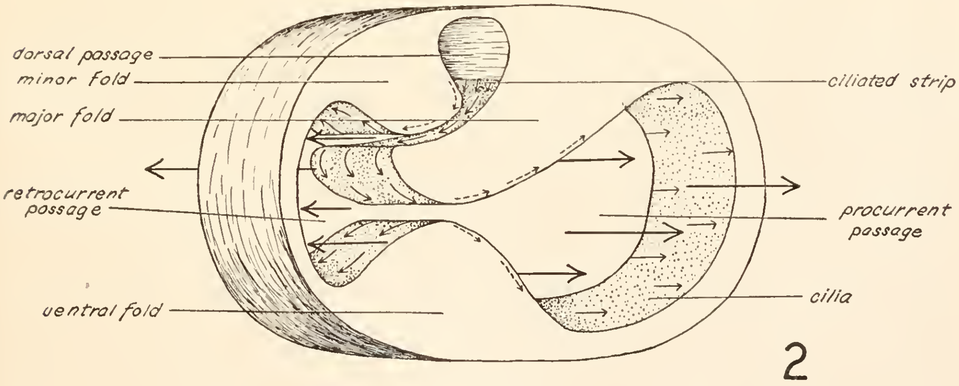
FIGURE 2. Stereogram of cross-section of the pylorus, taken midway between the gizzard and the hepatic vestibule. The stippled surfaces are heavily ciliated. (The small arrows indicate the direction of the ciliary beat; the large arrows, the direction of passage of material in the pylorus. The arrows with broken stems designate the direction of ciliary beat on surfaces behind the folds.) × 25.

ABBREVIATIONS

AT, atrial artery; *CC*, cecal artery; *d.p.p.*, dorsal pyloric passage; *GD*, dorsogastric artery; *HN*, minor hepatic artery; *HP*, prohepatic artery; *IP*, prointestinal artery; *m.p.f.*, major pyloric fold; *n.p.f.*, minor pyloric fold; *pc.p.*, procurrent pyloric passage; *PM*, major pyloric artery; *PN*, minor pyloric artery; *PP*, propyloric artery; *PV*, ventropyloric artery; *rc.p.*, retrocurrent pyloric passage; *v.p.f.*, ventropyloric fold; *VT*, vestibular vascular arborescence.

PLATE I


PV
HN
u.p.f.
pc.p.
rc.p.
m.p.f.
n.p.f.
d.p.p.
pp
PM
PN
pylorus
IP
anterior lobe of liver
typhlosole
prointestine
GD
IP
IP
atrium
AT
cecum
CC
VT
hepatic vestibule
hepatic ducts
HP
posterior lobe of liver
1


dorsal passage
minor fold
major fold
retrocurrent passage
ventral fold
ciliated strip
procurrent passage
cilia
2

ciliary currents pass out of the dorsal into both the procurrent and the retrocurrent passages. The major fold in addition bears a thin longitudinal strip of long cilia at its boundary with the dorsal passage. The major and minor folds in the living animal nearly always touch along their crests, so that the fluid contents of the dorsal passage may pass into the two ventral passages but coarse material from the ventral passage may not pass into the dorsal passage. The juxtaposition of the two folds is continued under the hepatic vestibule, where the folds provide a ventral floor to this chamber. At this point the cilia on the folds direct a powerful current out and away from the vestibule, again preventing the entrance of coarse material into the hepatic ducts and liver.

As discovered for *Helix* by Merton (1923), the corrugations of the larger proximal portions of the hepatic ducts of *L. s. appressa* bear two ciliary countercurrents (Fig. 11): the cilia on the crests of the corrugations are long and beat into the liver, those in the grooves are shorter and pass particles in the direction of the hepatic vestibule and into the incurrent tubule of the cecum. The particles in the grooves are quickly entrapped in mucus secreted there and formed into delicate strings. The currents directed into the liver could be traced with certainty only in the large hepatic ducts, although cilia were observed as far as the peripheral follicles in isolated bits of living liver tissue. Yonge (1936) states that in Mollusca where food passes into the liver and waste material out, the ducts are ciliated in such a way that an inward passage exists on one side and an outward one, on the other. Such counter currents could not be determined in *L. s. appressa*.

In the cecum the cilia on the cecal folds beat off the folds into the tubules (Fig. 9); those in the incurrent tubule pass carmine particles directly to the distal end and around this into the excurrent tubule. Here the cilia beat circumferentially, rotating the contents of the tubule along the longitudinal axis. In the continuation of the excurrent tubule across the pyloric wall the ciliary stream is directed towards the prointestine.

The crop, pylorus, liver, and hepatic ducts are as active as the postesophagus. Besides the usual peristaltic movements, they undergo a series of violent alternating pulsations, here designated *pulsatory movements*, in which the crop, pylorus, hepatic ducts, and liver pulsate successively, forcing the fluid contents slowly back and forth in swirling currents. In the pylorus the pulsations commence at a point between the typhlosole and the atrium and pass towards the gizzard. They are of two types: (1) very strong pulsations in which the entire structure contracts and (2) minor pulsations running over restricted portions of the pylorus. In the liver the pulsations pass as far as the terminal follicles. This marked movement is most vividly observed in bits of living liver tissue under high magnification. Individual cells are seen to move against each other by contraction of the thin muscular connective sheet enveloping each follicle. The pylorus undergoes the most pronounced movements and appears to lead the other organs in activity. The incurrent tubule of the cecum is relatively thin-walled and does not appear to undergo peristaltic activity. The excurrent tubule is thicker-walled and has definite peristaltic movement in the direction of the outlet.

It follows then that one of the important functions of the pylorus is that of a filter chamber, separating the digested and the fine, partly digested food particles from the gross material and sand. This is the conclusion which Heidermanns (1924) also reached when he stated that most of the time sand and gross material are kept from passing into the liver by the pyloric folds. The major and minor folds remain in close approximation along their crests, leaving a narrow slit between the dorsal and the ventral passages which may be called the *pyloric filter.* The cilia on the folds are well developed and beat away from the dorsal passage. During the pulsatory movements of the stomach region only the finest particles and fluid material are permitted ingress to the liver through this filter. The *pulsatory currents,* as these in the gut lumen may be named, are relatively strong and in their streaming between the sand particles and foot bits in the gizzard cavity dislodge large particles of food. Those which are carried into the pro- and retrocurrent passages and which are too large to pass through the pyloric filter, become entangled in the ciliary currents of the folds and are carried quickly back to the *left* side of the gizzard lumen by way of the retrocurrent passage. The particles carried into the crop on the forward streaming of the contents are soon entangled in the ciliary currents of the crop and conveyed to the *right* side of the gizzard lumen. Here, then, is a delicate adjustment by which the larger particles dislodged from the gizzard contents are equally redistributed for further grinding within the gizzard.

At certain intervals during the day the pulsatory movements appear to cease and a portion of the residual material and sand in the gizzard pass out through the procurrent passage to the prointestine. The propulsion of *gizzard strings* (Fig. 10), as these residues may be named, through the procurrent passage is very slow and mostly by cilia supplemented by slight peristalsis. Cilia were found active throughout all portions of the alimentary tract whenever opened; no cessation of ciliary activity (as occurs in some lamellibranchs during increase of CO_2 concentration) or reversal of beating was observed. During emission of the gizzard string, the large portion of the ventral pyloric fold which partly occludes the gizzard lumen flattens to enlarge the opening. As suggested by Howells (1942) for *Aplysia,* it appears that the shape and position of the pyloric folds in *L. s. appressa* are partly maintained by blood pressure in the sinuses.

To what extent digestion does occur in the postesophagus, crop, gizzard, and pylorus is questionable. As amylase from the liver and from the salivary glands, trypsin from the salivary glands and cellulase, at least, are present in the gut contents, some food may be partly hydrolized. Part of the remaining available food is reduced mechanically to particles small enough for ingestion by the digestive cells of the liver. The amebocytes of the gut also appear to aid in digestion. According to Heidermanns (1924) fats and carbohydrates are absorbed in the pylorus by the ciliated cells.

The pyloric filter permits only minute food particles to pass into the liver. Most of the radular teeth which are discarded continuously from the radula throughout the life of the snail (Carriker, 1943a) and grains of sand as large as 90 μ, by reason of the fact that they are considerably heavier than the lighter food particles of the same dimensions, are carried past the cilia by the force of the pulsatory currents. The larger free food particles, especially of lettuce, are very light and are readily barred by the cilia of the filter. In the proximal portions of the hepatic ducts, because of counter ciliary currents, only the finer particles that fall into the grooves of the corrugations can be carried towards the cecum; thus teeth and larger sand grains are held at this point by the ciliary currents of the crests of the corrugations until sufficient fecal material passes out of the liver to carry them with it.

Ciliation of the crests of the corrugations may play a small role in the conduction of food material into the follicles of the liver, but probably the principal conveyers are the pulsatory currents. Food in solution and in suspension is thus brought to all the internal surfaces of the liver follicles. Larger particles finding entrance through the filter and too large to remain readily in suspension appear to fall to the ductal epithelium. The smaller of these are soon propelled into the grooves of the corrugations. Liberal quantities of mucus are secreted there, trapping the particles in mucous strings which pass towards the cecum, coalescing as they advance into the larger grooves (Fig. 11). From the incurrent cecal tubule the mucoid strings pass around the distal end of the cecum into the excurrent tubule. There the material receives a further transparent layer of mucoid and cementing material and is rotated into a smooth cylindrical continuous string, here designated the *cecal string* (Fig. 10). This, partly by ciliary action and partly by peristalsis, then passes on into the prointestine across the atrium. In snails feeding on green lettuce the strings are a vivid green because of a heavy accumulation of bits of chlorophyll bearing bodies which become entangled in mucous strings in the hepatic ducts. In gastropods fed on a food containing carbon, the cecal strings are a dense black. In animals on a starvation diet, the cecum continues to pass out cecal strings, just as in the feeding animal, but the strings are a mucoid, transparent, milky-white color and much reduced in diameter. It thus would seem that the function of the grooves in the hepatic corrugations and of the cecum is to collect and eliminate those fine particles which pass through the pyloric filter but which are too large to be engulfed by the digestive cells and which are thus mechanically eliminated by a "supplementary filter." Cecal strings pass out continuously, apparently at the same uniform rate and without apparent interruption. They provide a kind of "time clock" by which the rate of passage of the gizzard strings and the residues from the liver can be compared (Fig. 10).

Explanation of Plate II

(All figures concern *L. s. appressa*)

Figure 3. Ciliation currents of the postesophagus, crop, gizzard, pylorus, hepatic vestibule, atrium, and anterior portion of prointestine. The tract has been slit ventrally and spread. × 6.

Figure 4. Irregular blue-green excretion bodies (in vacuoles) taken from the liver string. × 500.

Figure 5. Smooth blue-green, or brown, excretion bodies (in vacuoles) taken from the liver string. × 500.

Figure 6. "Signet" excretion body (in vacuole) appearing in the liver strings. × 500.

Figure 7. Clear nodules found in the liver strings which when pressed out under the cover slip display their crystalline nature. They dissolve in dilute HCl and seem very similar to the calciferous concretions of the vesicular cells of the connective tissue. × 500.

Figure 8. Indigestible residues from digestive cell (in vacuole), found abundantly in liver strings. × 500.

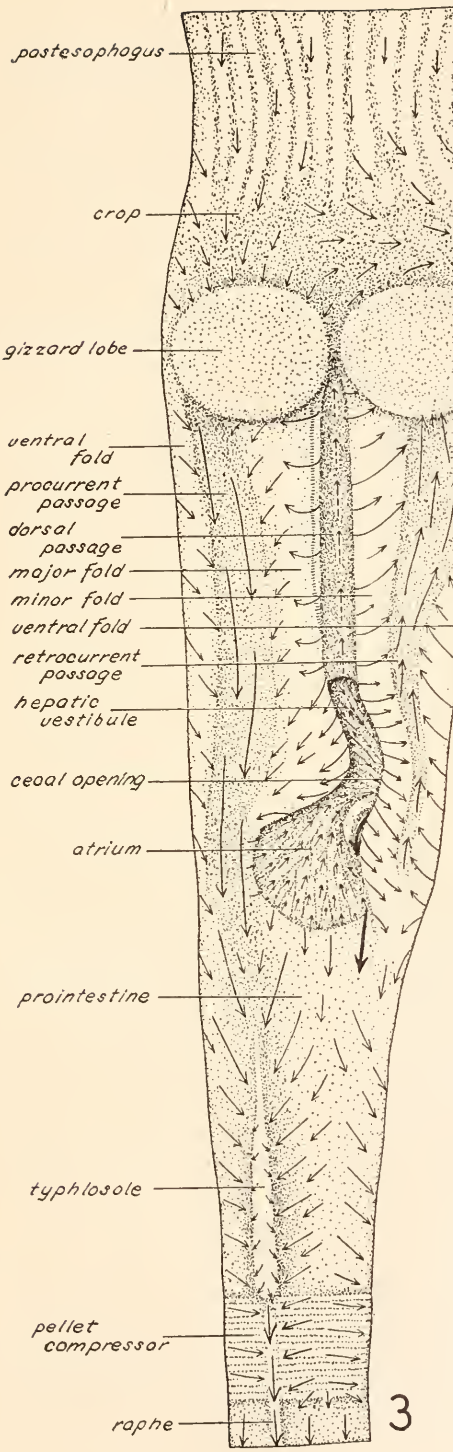
Figure 9. Ciliation currents of the cecum, which has been opened along the incurrent cecal tubule and spread flat. × 6.

Figure 10. Typical fecal pellet, showing the gizzard, liver and cecal strings, and the impression of the typhlosole in the pellet. × 6.

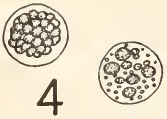
Figure 11. Portion of the corrugated epithelium of the hepatic duct, taken at the opening of the duct into the hepatic vestibule. (Large arrows indicate the direction of the ciliary currents in the grooves; the small arrows, that on the crests of the corrugations.) × 50.

Abbreviations

c.s., cecal string; *excur. tubule*, excurrent tubule; *g.s.*, gizzard string; *inc. tubule*, incurrent tubule; *l.s.*, liver string; *s.*, sand; *t.i.*, impression of typhlosole in fecal pellet.


postesophogus
crop
gizzard lobe
ventral fold
procurrent passage
dorsal passage
major fold
minor fold
ventral fold
retrocurrent passage
hepatic vestibule
ceoal opening
atrium
prointestine
typhlosole
pellet compressor
raphe

3



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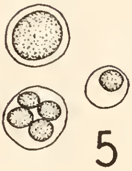

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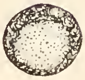

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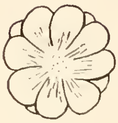

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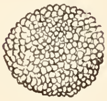

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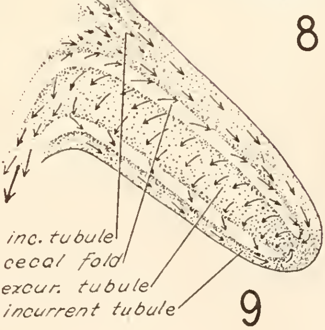
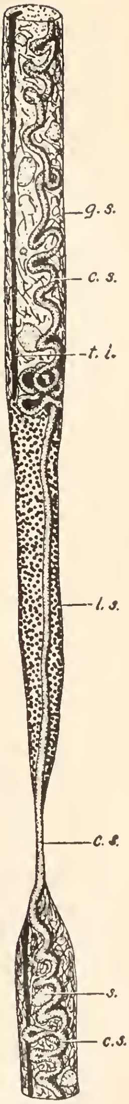
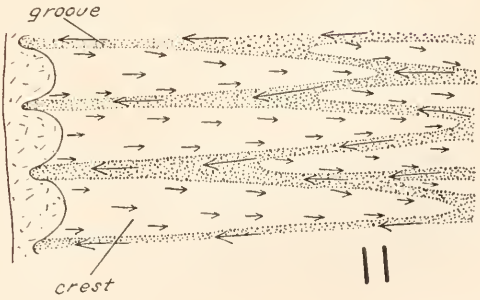

inc. tubule
cecal fold
excur. tubule
incurrent tubule

9


g. s.
c. s.
t. l.
l. s.
c. s.
s.
c. s.

10


groove
crest

11

The excretory bodies and indigestible residues in the liver are voided periodically. These are passed simultaneously in minute mucous strings from all parts of the liver towards the central hepatic ducts, there converging into larger strings which pass in the direction of the hepatic vestibule. At the proximal end of the hepatic ducts this material fills most of the main duct. The combined currents in the grooves of the corrugations appear to exert a stronger force than those on the crests, so forcing the waste material directly into the hepatic vestibule (Fig. 11). There it is caught by the outward flowing ciliary currents on the major and minor pyloric folds and passed rapidly into the prointestine. The excretory bodies and indigestible residues passing from both lobes of the liver are compressed in the hepatic vestibule into one bulky string which is distinct from the cecal and from the gizzard string and may be called the *liver string* (Fig. 10). It is drawn out of the liver at the same rate as the cecal string passes out of the ceum. Both strings are usually found parallel to each other and uncoiled in the fecal pellets. The gizzard string, on the other hand, passes out much more slowly so that the cecal string occurs loosely and abundantly coiled therein (Fig. 10). A lapse of time seems to occur between the exit of the gizzard string and that of the liver string, as indicated by a conspicuous coiling of the cecal string between the last portion of the gizzard string and the forward end of the liver string. The gizzard string follows the liver string immediately, as indicated by no noticeable coiling of the cecal string between the two. There is also some evidence that, as the liver string is drawn from the liver, the pulsations of the stomach region cease. In animals opened for physiological observation of the tract, the stomach region was never pulsating when the liver strings were passing out of the liver. This is desirable to prevent the dismemberment of the strings and their mixing with food material brought into the liver by the pulsatory currents. The merger of the strings in the prointestine produces the fecal pellets.

The pylorus is composed of a complicated system of folds and passages, it is innervated by a pair of complex nerve plexuses and a nerve net, and all of the parts are exceptionally well vascularized. Functionally there is present in this portion of the tract an intricate system of counter ciliary currents and synchronized muscular movements, as well as partial vascular control of the folds. The pylorus is thus well equipped to convey digestive fluids from the liver to the gizzard and crop, to bear digested and semi-digested particles into the liver from the gizzard, to exclude large sand and other large particulate matter from the liver and transfer such residues to the prointestine, to receive waste material from the liver and transport it to the intestine, to act in conjunction with the cecum, liver, and hepatic ducts in shunting a continuous string of residual particles from the walls of these organs into the prointestine, to secrete fluids (of unknown nature) and finally to absorb fats and carbohydrates.

Liver

The liver is probably the most important organ of digestion in the alimentary system of the gastropods. Peczenik (1925) shows, as has been indicated in this work also in feeding experiments, that such proteins as egg albumen are engulfed and digested intracellularly in the digestive cells, and the indigestible residues are cast out in vacuoles. Krijgsman (1928) believes that digestive cells in *Lymnaea* are also secretory as well as absorptive, as he has often observed numerous typical secretion granules in the liver cells of starved snails. Biochemical tests indicate that the greatest catheptic activity of the snail body is localized in the liver, yet none of this activity has been found in the fluid of the gut. This is in keeping with catheptic systems in other animals in which the enzyme has been shown to exist entirely as an intracellular protease. Hurst (1927) writes that in *Physa* fat and glycogen are stored in the digestive cells. Fat was also found in the lime cells of *Helix* by Grünbaum (1913). The problem of *what size* of food particle is engulfed through the distal membrane of the digestive cells is still an open question. It is likely, as indicated by the work of Krijgsman (1925, 1928) on *Helix*, that the lime cells function in storing and in periodically secreting a buffering agent which adjusts the pH of the gut juice; this point has not been investigated in *L. s. appressa*. The mucous cells of the liver provide the mucus utilized in the binding of the indigestible residues and the excretory bodies into the liver strings.

Amebocytes were found in varying numbers in the contents of the lumina of the liver, postesophagus, gizzard, and pylorus. These were similar to those seen in the blood. In some instances those in the gut contained fecal vacuoles so large as to force the cell into a peripheral lobate ring.

Rhythmic activity of the liver is suggested by inspection of sectioned liver tissue, of fecal pellets and of the living organ in various phases of its activity. Pulsatory movements of the stomach region are apparently interrupted only during the passage of liver strings and of gizzard strings. This may explain why smaller hepatic excretory bodies occur in the upper pylorus, gizzard, crop, and postesophagus in such insignificant numbers. If the pulsatory currents persisted during the elimination of the liver residues one would expect to find liver string detritus scattered over the gut in as great profusion as in the liver, along with the reddish colored secretions from the liver.

The inclusion bodies of the digestive cells of *L. s. appressa* have been studied in detail in the living cells of normally feeding snails, starved snails, snails fed on special diets and in preserved tissue sections. The egested bodies have been followed in the fecal pellets over a period of weeks. The results of the study clearly indicate the presence in the digestive cells of excretion bodies, of indigestible residues and of secretion in separate vacuoles.

Figure 8 illustrates a vacuole from the digestive cells which is filled with indigestible particles. These vacuoles measure 12 to 25 μ in diameter. In snails feeding on lettuce the contents are colored a greenish brown to dark brown and are composed of minute irregular particles, some of the larger ones of which measure about 3 μ in diameter. In the digestive cells they occur one per cell and in varying stages of particulate concentration. These constitute the bulk of the liver strings and retain their identity in fecal pellets which have been voided for several days.

The secretion granules are clearly evident in preserved histological sections stained with iron hematoxylin, especially grouped towards the distal area of the cell. Larger granules measure as much as 4 μ in diameter.

The excretion vacuoles (Figs. 4, 5, 6) when in the cells may measure as much as 25 μ in diameter, but in the fecal pellets have shrunk somewhat. In the living cells excretion bodies are found in variable form and color and are best observed when the cells are slowly pressed out under a cover slip as the fluids evaporate. The cell contents then pass rolling and turning from the ruptured cells, exposing the different surfaces of the inclusions. There is one series in which the vacuoles range from small to large vacuoles containing variable numbers and sizes of minute blue-green, translucent, many-angled particles. The smaller particles are in constant Brownian movement, dancing around like a swarm of bees, and indicating the low viscosity of the fluids in the vacuoles (Fig. 4). In a second series the same variation in size of the vacuole is encountered but the blue-green bodies are present in groups of only one to four per vacuole and are spherical and smooth (Fig. 5). In a third series the vacuoles and bodies are identical in form to the second series, but the color of the bodies varies from a light brown to a dark solid brown. The largest of these bodies are sometimes found free of the vacuoles. When compressed under a cover slip they spread with a flowing viscous movement, much as a drop of heavy molasses spreads when pressed between two smooth surfaces. In the fecal pellets these vacuoles are usually found varying in diameter from 3 to 15 μ, and the vacuole membrane presses closely around the excretion body. A fourth type of excretion body is found which varies in diameter from 12 to 18 μ, is colored a dark brown with a smooth center and possesses a periphery of irregular markings, such that the body resembles a signet ring (Fig. 6). The excretion bodies described above are present principally in the liver strings, and only in negligible numbers in the cecal strings. The "browns" and "signets," particularly, stain with methylene blue and neutral red and do not dissolve in strong HCl. The different types described are not all present in any liver string in equal abundance at any one time, but vary independently, in a sequence which did not seem significant. Because of the transitional stages between some of these excretion bodies it is probable that they are all different phases of the same type of metabolic excretion; but the method of their formation is still a puzzle.

Intestine and rectum

Cilia on the typhlosole beat towards the lateral sides of the typhlosole (Fig. 3); those over the prointestine around the typhlosole beat circumferentially and somewhat obliquely from the dorsal to the ventral sides in a symmetrical pattern. The division of the currents occurs along the dorsal line of the prointestine. Over the pellet-compressor the cilia beat transversely across the intestine. The raphe bears a strong current which streams directly posteriad. Thus in the pellet-forming region, through ciliation and muscular movement, loose particles are gathered, rolled inward about the typhlosole and folded into a compact pellet. Strong ciliary currents in the remainder of the intestine and rectum are limited almost entirely to the costae, raphe, and pseudoraphe; cilia of the intercostal surfaces are relatively short and weak. Peristaltic activity is evident throughout the intestine and rectum, being noticeably strongest in the early portions of the prointestine, just behind the pellet-compressor.

Abundant vascularization of the prointestine, in contrast to the relatively poor vascularization of the esophagus, suggests that this region of the intestine may also function in the absorption of food and water.

Consolidation of the cecal and liver strings occurs at the hepatic vestibule; of the gizzard residues and cecal string, in the pellet-forming region. The cecal string as it is moulded in the cecum is already a smooth well cemented string and undergoes no further change as it is forced continuously across the outer margin of the atrium.

The liver string, characterized by a fine dark brown mottling and almost as well concentrated as the cecal string, receives a final transparent envelope of cementing fluid which binds the cecal string to it (Fig. 10).

The chief function of the pellet-forming region is that of consolidating and cementing the loose straggling gizzard residues which constitute by far the greatest bulk of the fecal pellet. The large numbers of mucous cells, basophilic flask cells and basal secreting cells about the pellet-forming region are indicative of the large quantities of cementing substance secreted during the moulding of the pellets. By means of ciliary streams and constriction of the tube at the pellet-forming region the gizzard residues are pressed into pellets, and the cecal strings, lying loosely coiled in these residues, are simultaneously incorporated in the pellets. These are then forced out of the pellet-forming region by ciliary activity and by strong peristaltic movements which are noticeably stronger immediately behind the pellet-compressor. Peristaltic activity gradually diminishes in the direction of the anus. The conspicuous impression of the typhlosole remains in the fecal pellet, particularly in the gizzard string portion, as long after defecation as the pellet retains its form. Moore (1931) has found variable patterns in the fecal pellets of different Gastropoda and points out the importance of identification of animals by means of their pellets. A most striking fact about fecal pellet formation is the extreme com-

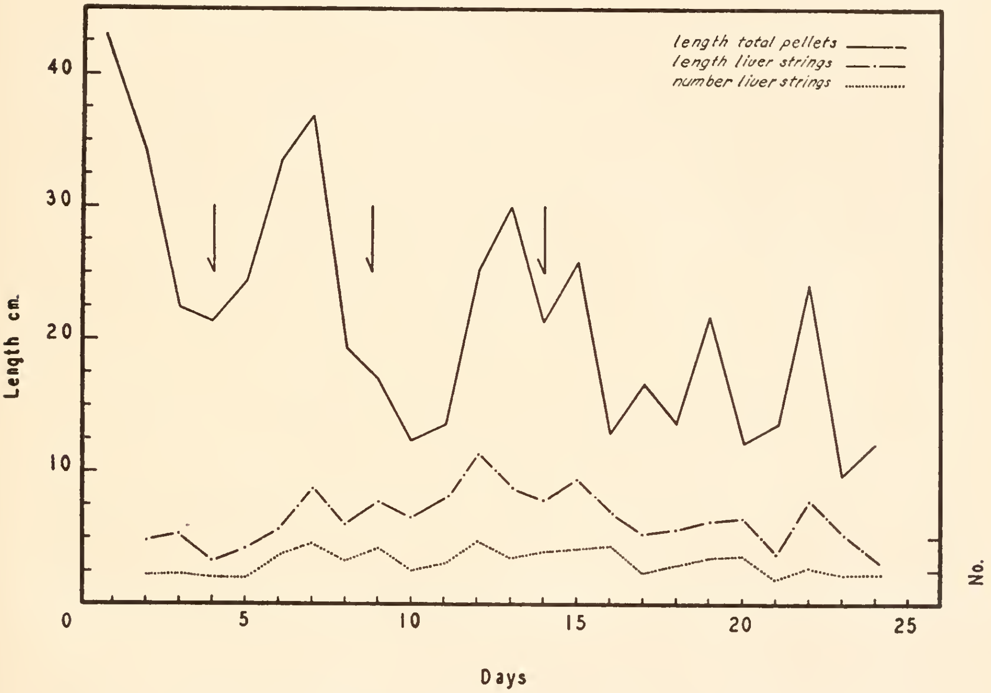


FIGURE 1. Length in millimeters of the liver and gizzard strings and number of liver strings of the fecal pellets, calculated on a twenty-four hour basis. These were voided in a period of twenty-four days by a forty millimeter *L. s. appressa.* The vertical arrows indicate the time at which egg masses were oviposited.

pleteness with which fecal material is compressed and cemented. This presumably prevents fouling of any portion of the tract.

For any given snail the diameter of the gizzard string portion of the fecal pellet is constant, varying principally with the size of the snail. The liver string varies in diameter from that of the gizzard string to that of the fecal string. Figure 1 indicates for a forty millimeter snail over a period of twenty-four days the rate and extent of voidance of fecal pellets. For the tabulation of this data the fecal pellets were collected daily and arranged end to end under the binoculars and measured to the nearest millimeter. The measurements given indicate only the lengths of the gizzard and liver strings, as the cecal string generally occurs embedded in the first two strings. The diameter of the gizzard string is reliably constant; that of the liver, less so.

Most conspicuous is the fact that the quantity of fecal pellets voided daily is quite variable from day to day. The quantity of gizzard strings fluctuates far more erratically than does that of the liver strings, indicating that the volume of material utilized by the liver is more constant than that which may pass through the gizzard. The number of liver strings is a more conservative indicator than the length of strings, and is probably not as accurate. Passage of food through the gizzard, and thus food consumption, seems to diminish during oviposition.

As indicated by the following data, feces were voided in about equal quantity day and night, with just a slight daily increase, over a period of twenty days (9 P.M. to 9 A.M., and 9 A.M. to 9 P.M., respectively):

Pellets	Night	Day
Total length of pellets, mm.	1,987	2,134
Total length of liver strings, mm.	530	588
Total number of liver strings	110	113

The total length of fecal pellets passed in the twenty-four days was 5,645 mm.; and the total length of liver strings, 1,491 mm., was passed in 289 liver strings, giving an average length of 5.1 mm. per liver string. Actually the liver strings varied in length from one to 10 mm. The average calculated length of fecal pellets passed in twenty-four hours was 235 mm.; of liver strings, 62 mm. In a normally feeding snail the sequence of the liver strings with the gizzard strings was always one of alternation. Liver strings do not generally mix with the gizzard strings. Gizzard strings as long as 52 mm. were found connecting liver strings. Three typical series of fecal pellets taken from days one, two, and three on Figure 1 are given below. The liver and gizzard strings are represented by the lengths in millimeters of the strings in the order of their elimination; the figures for lengths of the gizzard strings are italicized. The total time for elimination of the pellets is given to the right in parenthesis:

(1) 6 *40* 7 *33* 7 *11* 6 *44* 5 (5 hrs. 15 mins.)
(2) *48* 7 *52* 8 *13* 4 *50* 5 *38* 6 (10 hrs. 15 mins.)
(3) *20* 7 *8* 7 *22* 6 *29* 9 *24* 3 (10 hrs. 30 mins.)

As indicated by the curve for total fecal pellets in Figure 1 and by the lengths of the gizzard strings in the series above, consumption of food appeared to follow an alternating heavy and light cycle.

In snails deprived of food the elimination of the gizzard strings ceased and liver strings then became connected only by slender lengths of cecal strings. When starvation had continued for ten or more days nothing but delicate white cecal strings and a few much reduced liver strings containing metabolic excretion bodies were found in the intestine.

A. H. Rosenbloom (unpublished bachelor's thesis, 1942) by feeding colored food to *L. s. appressa* at different times through a period of a month found that in normally feeding snails of approximately forty millimeters shell length the minimum time for the passage of food from the mouth to the anus was two hours and twenty minutes; in snails previously starved for a week, five hours and fifty minutes. He found also that previously starved snails feed for a longer consecutive time than do normally feeding snails. The present investigation shows clearly that the alimentary system becomes completely emptied of food a few days after starvation commences. Considerably more food and a longer time are required for a starved animal to fill the alimentary tract with food to the point where fecal material is voided than for a normally feeding snail.

The rhythm of passage of liver strings is in keeping with the rhythm of the liver itself in which all digestive cells appear to assimilate food together and discharge indigestible residues simultaneously. This cycle, as indicated by the passage of liver strings, is not completely unvarying, because the number of liver strings discharged daily varied approximately from eight to nineteen. Thus the interval between the discharge of liver residues, probably the time during which the liver was digesting food, varied in this experiment from seventy-five minutes to three hours. It is possible that oviposition (Fig. 1) may account for some of the variability.

There seems to be nothing in the literature concerning fecal cycles in the Gastropoda. Some few scattered observations are reported on the length of the fecal pellets. For example, Heidermanns (1924) writes that a 48 mm. *L. stagnalis* with a 90 mm. intestine, eliminated 120 mm. of feces in 24 hours.

The long intestine is characteristic of the herbivorous snail nutrition of *L. s. appressa*. One of the most striking facts about the functioning of the alimentary system is the meticulous care with which all loose particles are collected and properly disposed of, in this way serving as a highly efficient sanitation system. The fecal pellets receive additional external layers of cementing material as they pass down the length of the intestine and rectum. The pH of the intestine is slightly more alkaline than that in the stomach region. As pointed out by Yonge (1935) mucus is an amphoteric protein whose viscosity is augmented by higher pH, thus more efficient consolidation of the feces occurs. Elimination of the fecal pellets through the anus is a fairly rapid and uniform process. The strong anal sphincter muscle remains tightly contracted except during defecation. Fecal pellets, being slightly heavier than water, settle slowly to the bottom of the aquaria. The marked efficiency of the mucoid coating over the feces is indicated by the extended period after defecation that pellets retain their identity. Thus it would seem that the alimentary system has not only become specialized in the maintenance of hygienic conditions within the system, but also in furthering a healthy external environment.

Fecal pellets are ingested by snails even in the presence of fresh food and the animals appear to derive some nourishment from them. It is to be recalled that the gizzard is not a thoroughly efficient grinding mechanism and in many cases, particularly in the absence of sufficient fine sand, considerable unused available food passes out in the gizzard strings.

Discussion

The question as to whether the radula slides over the cartilage independent of cartilage activity has been a favorite point of academic controversy with certain malacologists for some time (in Lymnaeidae see Geddes, 1879; Amaudrut, 1898; and Pelseneer, 1935; in the Stenoglossa, a review: Carriker, 1943b). In *L. s. appressa* (and possibly in the majority of snails carefully investigated) there is no question but that the principal activity of the radula is that effected by the action of the cartilage and muscles under it, and a sliding of the total radula over the cartilage independent of the movement of the cartilage.

A study of the movements of the gut in *L. s. appressa* suggests that rather than the presence of different pH in the different portions of the gut, the pH may vary with the rhythms and secretions of the liver, the secretions of the salivary glands, the secretions of the unicellular glands of the gut wall and with feeding. It is quite unlikely that with the constant mixing of the gut contents as a result of the pulsatory movements at certain periods, the pH would vary markedly in the different lumina of the tract at any time. The wide range obtained between the maximum and the minimum pH's and the insignificant variation of the maximum and of the minimum pH's is in keeping with this suggestion. The partial isolation of the intestine from the movements of the stomach region is in keeping with the slightly higher pH found in the intestinal lumen.

The complexity and abundance of nervous tissues about the stomach region suggests a possible nervous control of the movements of the stomach region and of the liver. In its muscular structure there is no doubt that the buccal mass is the most complex organ in the alimentary system; functionally it appears that the region in and about the pylorus is the most intricate. The dense ramifications of blood vessels, the presence of two nerve plexuses, the intricate series of folds and the complicated ciliary streams in this region lend credence to this postulation.

Heidermanns (1924) has opened the question of the function of sand in the basommatophoran gizzard in his comparative study of *Ancylus, Planorbis, Physa, Lymnaea* and certain stylommatophorans. He points out that in land pulmonates the flaring portion of the esophagus is called the stomach, whereas in the aquatic pulmonates the esophagus is normal and the stomach has become differentiated into the crop, gizzard and pylorus. Thus the Stylommatophora have no organs that could properly be homologized with the stomach of the Basommatophora. The gizzard and, with few exceptions, sand in the tract are absent from the land pulmonates. The gizzard, he states, reaches its peak of specialization in *L. stagnalis* and probably rose by reason of the ingestion of sand with food. He observed that in all Basommatophora the gizzard originates in front of the first flexure of the gut, apparently as a muscular band whose primitive function was to dispose of sand masses tending to congest there. This primitive type of gizzard is exemplified by that of *Ancylus* and the intermediate type by that of *Planorbis*. Heidermanns in support of his theory of the origin of the gizzard through a specialization of a primordial portion of undifferentiated gut, attempted to show modification of the gizzard in one snail generation by the use of various diets. As might be anticipated, he got no significant structural changes.

The fact that *Lymnaea* possesses the gizzard grinding mill may explain the observation stressed by Heidermanns that the cellulase of this snail is less active than that of *Helix* which has not developed a gizzard and consequently needs a strong cellulase for the hydrolysis of the cell walls of plant food consumed.

There is striking similarity in the functioning of the alimentary tract of the herbivore *Onchidella celtica*, ably presented by Fretter (1943) in a recent paper, and that of *L. s. appressa*. Perhaps this similarity is not to be wondered at when, as Fretter writes, "Many of the features which the Onchidiidae share with the pulmonates may be attributed to the close origin of the two groups, the similarity of their diet and their air-breathing habit."

SUMMARY

1. A balanced physiological salt solution was developed which maintains contractions of the vas deferens for approximately 66 hours.

2. Cathepsin was found in greatest concentration in the liver and no activity could be ascertained in the gut fluids. Some trypsin was indicated in the salivary glands. Amylase showed greatest activity in the salivary glands and the liver.

3. Muscular activity of the alimentary system involves the manipulation of the mouth parts in the buccal mass, peristalsis in the remainder of the tract, marked pulsatory movements of the postesophagus, crop, pylorus and liver, and a kneading motion of the gizzard. The radula is moved principally by the action of the odontophore but also operates independently of it.

4. The entire alimentary system, with the exception of the gizzard and parts of the buccal cavity, is ciliated. The cilia show definite directional streams which function in propelling food particles, in sorting food and in consolidating fine refuse particles with the aid of mucoid substances.

5. Sand is consumed normally by the snail and is necessary for the proper functioning of the gizzard in the crushing of food particles. Very little trituration is performed by the mouth parts.

6. The pylorus is composed of a complicated system of folds and passages and counter ciliary currents and functions as a filter which permits only the soluble and the finer food particles to pass into the liver. It shunts the undigested residues from the gizzard into the prointestine.

7. In the liver the digestive cells function in secretion, assimilation, intracellular digestion and excretion. The indigestible foods and the excretory products, as variably shaped and colored inclusion bodies, are eliminated in vacuoles.

8. The cecum functions in collecting the finer residues from the liver and forces them in a continuous string into the prointestine.

9. The residual material coming from the gizzard, liver and cecum is characteristic for each organ and is readily identified as distinct in the fecal pellet.

10. The prointestine is specialized in the final consolidation of gizzard, liver and cecal strings with the aid of cementing substances secreted by the basophilic flask cells and the basal cells.

11. The rhythmic nature of the liver is disclosed principally by a study of the fecal pellets.

12. *L. s. appressa* is an herbivore. Food bits are cut away by the radula and swallowed. In the buccal cavity the food receives mucus from the buccal gland

cells, mucous cells and the salivaries and enzymes from the latter. Temporary storage and initial digestion occur in the postesophagus. Digestive fluids pass up from the liver in the pulsatory movements of the stomach region which keep the fluid gut contents in constant circulation. The crop, gizzard and anterior portion of the retrocurrent passage of the pylorus comminute the food. Amebocytes present in the gut contents appear to aid in digestion. Soluble and fine particles of food pass through the pyloric filter into the liver where it is assimilated by the digestive cells. Assimilation also occurs in the pylorus and absorption possibly in the intestine. There is some evidence that the pulsatory movements of the stomach region cease during the passage of the gizzard and the liver strings.

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TEMPORARY PAIR FORMATION IN PARAMECIUM BURSARIA [1]

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In *Paramecium bursaria,* the two members of a conjugating pair normally remain united twenty to thirty-eight or more hours. During this time various nuclear processes take place, including three pregamic divisions, exchange and fusion of pronuclei, and three post-zygotic divisions. Clones that are capable of undergoing normal conjugation as described above are called "normal clones."

But there are some clones of this species which are abnormal [2] in that when they are mixed with normal clones the pairs formed are not lasting but separate within a few hours. An examination was made of such temporary pairs in order to discover what nuclear or other changes occur in them.

Fourteen clones of *Paramecium bursaria*, all belonging to Variety I, have been used in the present study. These clones, all of which were collected in nature, are listed in Table I with data on each clone including (1) the mating type to which it belongs and (2) the locality where it was collected.

TABLE I

Clones of Paramecium bursaria *employed in study of temporary pair formation*

Clone number	Original designation of clone	Mating type	Locality collected
1	SAa5	A	Santa Ana River, Cal.
2	Or3	A	Vicinity of Capistrano, Cal.
3	SGa3	A	San Gabriel River, Cal.
4	BG35	A	Los Angeles, Cal.
5	La3	B	Laguna Canyon, Cal.
6	SAa7	B	Santa Ana River, Cal.
7	UC13	B	Los Angeles, Cal.
8	BH2	B	Beverly Hills, Cal.
9	SAa4	D	Santa Ana River, Cal.
10	LP10	D	Lone Pine, Cal.
11	UC14	D	Los Angeles, Cal.
12	SAa1	C	Santa Ana River, Cal.
13	BH7	C	Beverly Hills, Cal.
14	BH101	C	Beverly Hills, Cal.

Clones 1–11 are normal clones in that they are capable of undergoing normal conjugation. Clones 12–14 are abnormal clones in that when they are mixed with normal clones the pairs formed are not lasting but separate within a few hours.

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[1] This work was aided by grants from the Committee for Research in Problems of Sex, National Research Council; and from the Joseph Henry Fund of the National Academy of Sciences.

[2] These clones are considered abnormal here only because they are incapable of taking part in the formation of lasting pairs when they are mixed with other, normal clones.

The animals were cultured in essentially the manner described by Jennings (1939). For cytological study the animals were fixed in Schaudinn's fluid containing glacial acetic acid, stained in iron hematoxylin, and destained in saturated aqueous solution of picric acid, following the technique the writer has described (Chen, 1944).

Experimental Studies

Most of the present work was done with the two abnormal clones (BH7, SAa1), although some study was also made on a third abnormal clone (BH101). All of these three abnormal clones belong to mating type C of Variety I. These abnormal clones were mixed with normal clones. Eleven such normal clones (all belonging to Variety I) were used. Four of these normal clones belong to mating type A; four to type B; and three to type D (see Table I).

As an example of the phenomenon of temporary pair formation, the reaction between the abnormal clone SAa1 and the normal clone UC13 will be described. On November 27, 1940, a large number of animals belonging to each of these two clones were mixed at about eleven o'clock in the morning. Strong agglutinative mating reaction occurred almost immediately. Half an hour after mixture, pairs were being formed. An hour after mixture (about noon) many pairs were formed. But in the early afternoon the pairs broke apart into single animals. By five o'clock all but a few pairs had separated. By evening all had separated.

Such temporary pair formation was also observed when the abnormal clone SAa1 was mixed with the following normal clones: Or3, SGa3, SAa5, La3, SAa7, BH2, SAa4, LP10, and UC14; or when the abnormal clone BH7 was mixed with the normal clone LP10; or when the abnormal clone BH101 was mixed with the normal clone BG35.

If such a mixture was placed in a moist chamber and kept from drying (with occasional replacement of the fluid that evaporated), the typical agglutinative mating reaction and temporary pair formation recurred the following day and almost daily over a period of many days. Some such mixtures were kept under daily observation over a period of nineteen days. The following is the characteristic daily behavior of the animals in such a mixture. The agglutinative mating reaction occurs in the late morning. By noon, many pairs are formed. These pairs persist for a few hours. Between four and six o'clock in the afternoon only a few pairs are found. In the early evening one or two pairs may remain; none can be found after nine o'clock in the evening.

Cytological Studies

Nuclear conditions in the clones that form only temporary pairs

The writer has made a cytological study of twenty-one abnormal clones, including the two clones BH7 and SAa1, and nineteen of the twenty-two such clones described by Jennings (1944). It was found that fifteen of these clones possess micronuclei, while six appear to be amicronucleate.

Thus the amicronucleate condition is not the general cause of the peculiar behavior of these abnormal clones. It is probable that the persistence of the amicronucleate condition is a *result* of the inability to conjugate and acquire a micronucleus, rather than the cause of it. Apparently there are conjugating and non-conjugating races of amicronucleate ciliates. In nature those that can conjugate do so and acquire a micronucleus, leaving in the amicronucleate condition only those incapable of conjugation. In my experience with *P. bursaria*, which includes a study of the nuclei and chromosomes of many clones (collected from different parts of the United States, Canada, Russia, England, Ireland, and Czechoslovakia), the only amicronucleate animals found in nature are those which cannot conjugate. Since they cannot conjugate, it is likely that such clones will be permanently amicronucleate. In nature any amicronucleate animal that can conjugate would not remain amicronucleate for long, since it would become micronucleate after mating with a normal animal from whom it receives a pronucleus as a result of conjugation (Chen, 1940).

Amicronucleate animals that can conjugate have been found in *P. bursaria* (Chen, 1940) [3] and in *Euplotes patella* (Kimball, 1941). They arose spontaneously in laboratory cultures.

Nuclear changes in temporary pair formation

To determine whether nuclear changes occur in temporary pair formation, a series of preparations were made, in December, 1940, of temporary pairs (abnormal clone SAa1 × normal clone UC13) [4] and a number of separated animals belonging to the latter clone. The material included pairs 5 to 6 hours after onset of temporary mating, separated animals a few hours after separation, 13 to 17 hours after separation, and 21 hours after separation. The micronuclei in these temporary pairs and separated animals were compared with the micronuclei of vegetative animals of clone UC13 (not mixed with any other clone). It was found that micronuclei in the majority of the temporary pairs and of the separated animals were slightly swollen. In some, no nuclear changes were apparent.

In June, 1943, a series of preparations were made of temporary pairs (abnormal clone BH101 × normal clone BG35) [5] and a number of separated animals belonging

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[3] The writer has recently found some additional cases of conjugation between amicronucleate and normal animals in *Paramecium bursaria*, in Variety III. (Normal nuclear changes occur in the conjugants having the micronuclei.) These amicronucleate animals arose spontaneously in laboratory cultures.

Schwartz (1939) in a brief preliminary paper reported "conjugation" in *Paramecium bursaria* between amicronucleate and normal animals and between two amicronucleate animals. In view of the lack of details in this report, it is impossible to tell whether temporary or lasting pair formation took place.

[4] Clone SAa1 appears to be amicronucleate; clone UC13 has a deeply staining micronucleus.

[5] Clone BH101 has a small, lightly staining micronucleus; clone BG35 has a relatively large, deeply staining micronucleus.

EXPLANATION OF FIGURES

FIGURES 1–34. Micronuclei of animals belonging to clone UC13 before, during, and after temporary pairing with animals of clone BH7 (drawn by Mr. Earl Nielsen). All drawings were made with a camera lucida. × 3,300.

FIGURES 1–5. Resting micronuclei of vegetative animals.

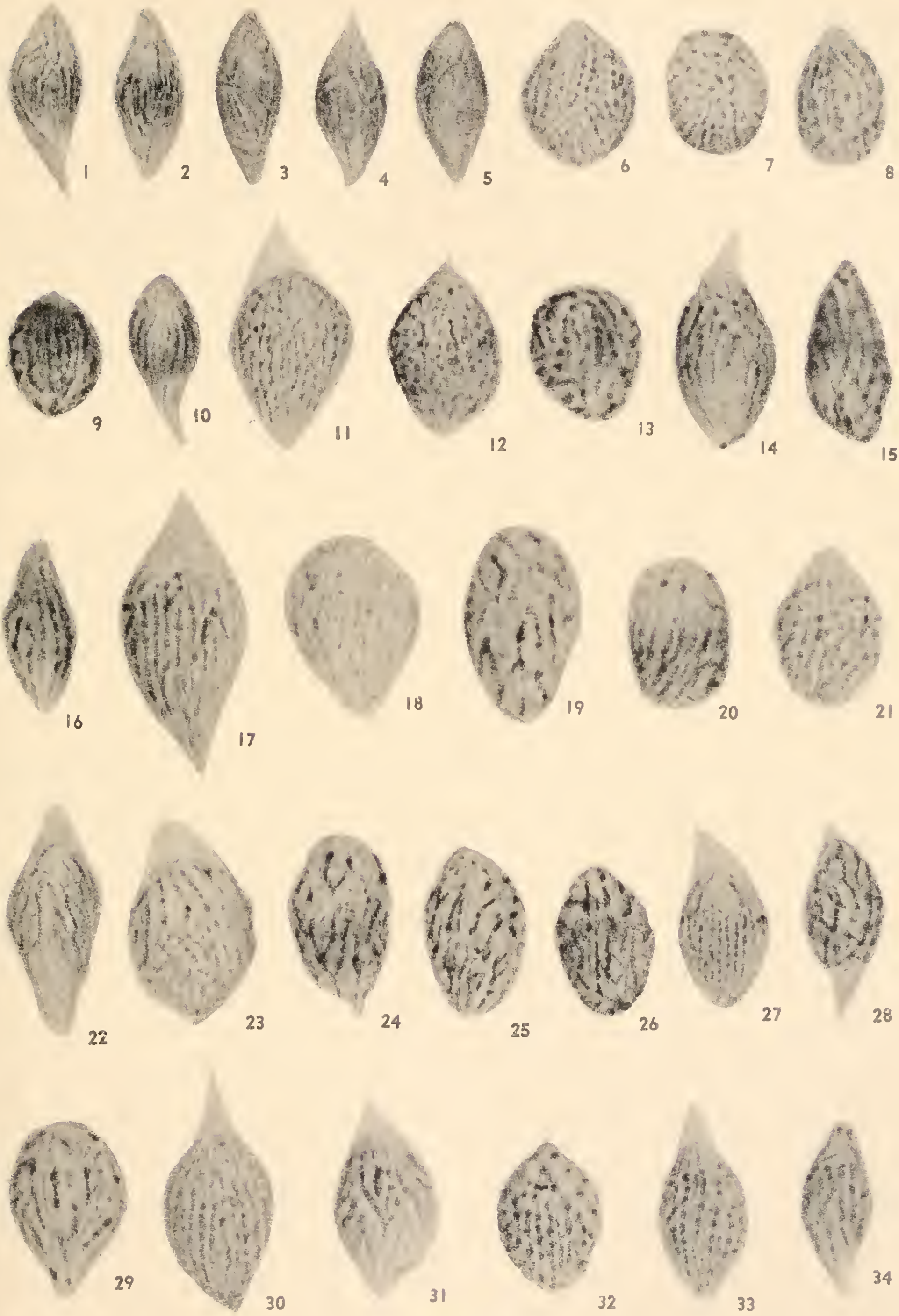
FIGURES 6–10. Micronuclei in the members of temporary pairs 4 hours after onset of pairing.

FIGURES 11–16. Micronuclei in the separated animals 18 hours after separation.

FIGURES 17–22. Micronuclei in the separated animals 30 hours after separation.

FIGURES 23–28. Micronuclei in the separated animals 42 hours after separation.

FIGURES 29–34. Micronuclei in the separated animals 51 hours after separation.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Figures 1–34.

to the latter clone. The material included pairs 3 to 4 hours after onset of temporary pairing, and separated animals 2 hours after separation, and a day after separation. It was found that the micronuclei in the majority or most of the temporary pairs and separated animals were slightly swollen. In others no nuclear changes were apparent.

In October, 1944, a series of preparations were made of the temporary pairs (abnormal clone BH7 × normal clone UC13) [6] and separated animals belonging to the latter clone. The material included pairs 4 hours after onset of temporary mating, and separated animals 7 hours after separation, 18 hours after separation, 30 hours after separation; 42 hours after separation, 51 hours after separation. A series of preparations of vegetative animals of clone UC13 (not mixed with any other clone) were used as controls (Figs. 1–5). It was found that the micronuclei in nearly all of the temporary pairs and separated animals were slightly but noticeably swollen (Figs. 6–34). This is true even of the separated animals 51 hours after separation (Figs. 29–34), indicating that the physiological effect of the contact between the animals in temporary pairing (as shown by the swelling of the micronucleus) is of long duration.

General Relations

The temporary pair formation described in the present paper is similar to that reported by Sonneborn (1942) in *P. aurelia* and by Jennings (1944) in *P. bursaria*. Sonneborn (1942) concluded from his data that cell adhesion occurring in the initial stage of the mating reaction and cell fusion occurring during subsequent conjugation are due to two different mechanisms.

Summary and Conclusions

1. In normal conjugation of *Paramecium bursaria,* the two members of each pair remain united for 20 to 38 or more hours, during which time various nuclear processes take place including three pregamic divisions, exchange and fusion of pronuclei, and three post-zygotic divisions. Clones that are capable of undergoing normal conjugation as described above are called "normal clones."

2. Some clones of this species are abnormal in that when they are mixed with normal clones the pairs formed are not lasting but separate within a few hours.

3. In temporary pair formation, the animals of diverse mating types when mixed exhibit the typical agglutinative mating reaction. Within an hour many pairs are formed but in a few hours these pairs break apart into single animals.

4. If such a mixture is placed in a moist chamber and kept from drying (with occasional replacement of fluid that evaporates) such agglutinative mating reaction and temporary pair formation will recur daily over a period of many days.

5. Cytological study of 21 such abnormal clones shows that most of these clones have micronuclei; some appear to be amicronucleate. Therefore, amicronucleate condition cannot explain the incapacity for taking part in the formation of lasting pairs. It is probable that the persistence of the amicronucleate condition is a *result* of the inability to conjugate and acquire a micronucleus rather than the cause of it.

6. In temporary pair formation, there are no conspicuous nuclear changes either in the pairs or in the animals after their separation. In the majority of the tempo-

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[6] Clone BH7 appears to be amicronucleate.

rary pairs and separated animals, there is, however, a slight swelling of the micronuclei. This swelling persists for a considerable length of time after the separation of the animals, indicating that the physiological effect of the contact between the animals in temporary mating (as shown by the swelling of the micronuclei) is of long duration.

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THE SPACE-TIME PATTERN OF SEGMENT FORMATION IN ARTEMIA SALINA

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INTRODUCTION

The present work was carried out in an attempt to arrive at a primary understanding of the regularities and laws in the phenomenon of metameric segmentation, as related to the shape and size of animals. To date this phenomenon, although of widespread occurrence amongst the higher animal phyla and thus probably an integral part in the more complex patterns of evolutionary organization, was nevertheless surprisingly rarely, if at all, subjected to analytical inquiry. The reason for this can probably be found in an essential lack in the past of well-defined concepts about the interrelations between mass, shape, growth, and degree of development of living organisms. The problem of segment formation in relation to size and shape is primarily one involving a clear appreciation of the dynamic geometry of living matter, and initial insight into the problem can therefore only emerge from rigorous observation on a quantitative level, followed preferably by geometrical and mathematical analysis. Such a method has been employed in the present work, and the results gained are conclusive enough not only to point the way for further study of the problem at hand, but also to promise reasonable success in the application of the quantitative, geometrical method to questions of biological space-time pattern in general.

The choice of Artemia has proven particularly fortunate for a study of metameric segmentation. The animal, held to be amongst the most primitive of living Crustaceans (Lockhead, 1941), develops few, if any, specialized structural features which would ordinarily tend to obscure the fundamental processes of morphogenesis. Moreover, the development of as many as nineteen body segments, a further primitive trait, is of obvious advantage in the investigation of the underlying principles of formation. Also, Artemia is easily obtained and can be reared in the laboratory without difficulty.

METHODS AND MATERIALS

Larvae of *Artemia salina* were obtained from commercial, air-dried egg cysts. Since excystment is retarded or inhibited in water above a certain salinity (Jennings and Whitaker, 1941), water of a specific gravity of 1.020 was used throughout as the initial medium. The egg shells cracked open usually 12 to 18 hours after contact with the water, and emergence of the larvae (Whitaker, 1940) took place between 18 and 24 hours. Portions of five stock solutions of brines with different salt concentration were employed as further media. The solutions were obtained from the original sea water by either diluting with doubly glass distilled water or concentrating with NaCl to specific gravities of 1.022, 1.033, 1.047, 1.066, and 1.085, respectively. All solutions were vigorously aerated daily, and possible deviations from the proper specific gravity were adjusted in weekly intervals. All work was carried out at room temperature, corresponding to an average water temperature of 21–22° C. As soon as the embryos had emerged, still enclosed within the fine hatching membrane, they were transferred to water from either of the five stock solutions. The moment of hatching, occurring within 24 to 30 hours after the cysts had first made water contact, was taken as zero time for all further determinations.

For observation the larvae were reared singly, in heavy crystal watch glasses. In the course of several observational series, a total of up to 100 individuals were observed, at least for certain periods of their development; of the 100, about 25 individuals, evenly distributed among the solutions, were reared from hatching to the adult stage. The presence or absence of a molted shell, the time, the temperature, the stage of development reached, and a series of measurements on bodily proportion were recorded for each individual twice daily in the earlier stages and daily for later stages. The animals were fed once every two days on a sea-water-yeast suspension. Each watch glass containing an animal was covered so that evaporation was nearly abolished, but a minimum of air circulation was always allowed for to equilibrize the CO_2 released by the yeast and the animal. The water was changed at two-day intervals for the younger stages and daily for older ones.

Larval body measurements were taken under the microscope with the help of a hemocytometer slide whose grid allows the direct reading off of lengths of 50 micra and consistent estimations of lengths of 10, 20, and 30 micra. If the larva is placed on a coverslip with a minimum of water, the whole can be adjusted in relation to the grid; evaporation is sufficiently slow to allow five or six measurements at a time. The error inherent in this method, viz., the parallax due to the thickness of the coverslip, is small enough to be negligible; also, since all measurements were taken in this way, the relative values are consistent.

PRELIMINARY OBSERVATIONS

Barigozzi (1939) and Rugh (1941) observed the total developmental time from hatching to the adult stage of Artemia to be 3 to 4 weeks. This is true as a broad generalization, but with the egg cysts in the various salinity media here employed, certain statistically preferred tendencies become apparent, expressed empirically by

$$D_x = D_0 \cdot S_x^{-6.8}$$

where D_x is the time, in days, for complete development from hatching in a solution of salt concentration x; D_0, the (hypothetical) time, similarly, for development in distilled water (the algebraic value turned out to be 36.55); and S_x, the specific gravity of the solution of concentration x. This relation holds good only in a statistical sense, within a specific gravity range of 1.020 and 1.1; it indicates that with higher salt concentrations the rate of development tends to be greater (Fig. 1). Irrespective of concentration a sigmoid curve of growth is always obtained.

Morphologically, different salinities have no differential effect on relative body proportions, a result to be expected in view of the conclusions of Bond (1932). An inverse relation between total size and salinity, observed by Bond, Heath (1924), and Warren (1938) for larvae from non-excysted eggs in the natural habitat, however, could not be observed for the excysted larvae here used; in the latter, total sizes are identical at equivalent stages of development, irrespective of salinity.

The number of molts between hatching and sexual maturity is not constant. Even when reared in the same medium, slight differences in molting frequency between several larvae may occur. Moreover, there exists a rough statistical relation between salinity and the total number of molts, approximating closely the above relation between salinity and the time required for complete development; in general, however, the number of molts for a given salinity is somewhat lower than the number of days required for development. In larvae from excysted eggs and under artificial food conditions, a range of 12 to 16 molts was observed between hatching and maturity, at a specific gravity of 1.085; this compares with 25 to 29 molts at a specific gravity of 1.022, and gradually decreasing molting frequencies for the intermediate salinity ranges. A staging of larval development according to molts, as Heath has done for non-excysted individuals, would therefore not be possible in the present case. Heath's 13 stages would hold for excysted larvae only when reared in brine of a specific gravity of 1.085; even then certain definite differences in the degree of development of equivalent molting stages can be

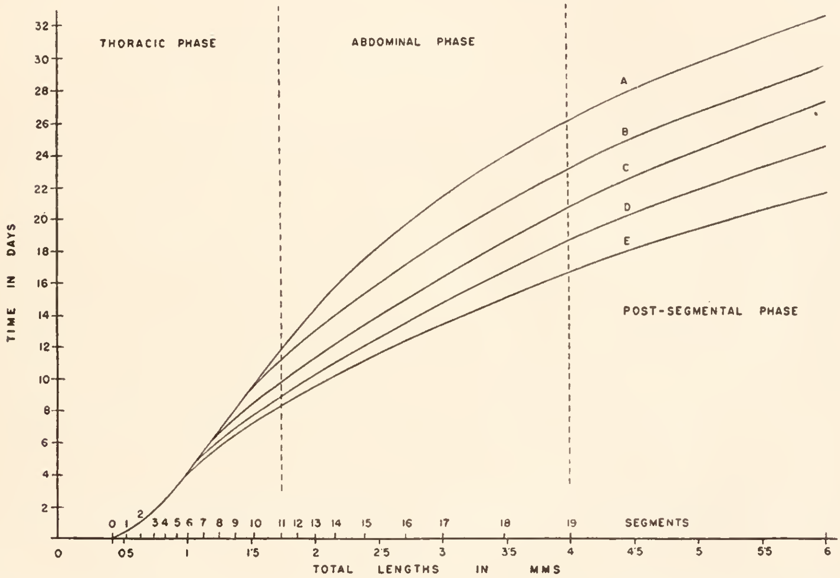

FIGURE 1. The effect of salinity on developmental time, from hatching to sexual maturity; absolute growth curves. Abscissa: total larval length; Ordinate: time in days. *A–E*, media of salt water, specific gravities from 1.022–1.085 respectively; numbers 1–19 above abscissa refer to number of body segments present.

observed, as comparison of Heath's descriptions with those below makes apparent.

With increasing developmental age the duration of instars increases; a 12 to 24 hour interval between molts in the younger stages compares with 24 to 30 hour intervals in older ones. The two factors of salinity and developmental age also determine the size increase between molts; for higher salinities, as well as for older larvae, the size increase is greater. There is no observable relation however between the time at which a molt occurs and the size or the developmental stage attained, irrespective of whether test larvae are reared in the same or at different salinities. Molting is also greatly influenced by the food supply. Starving animals do not molt; after 3–5 days an abortive attempt at molting is made which usually results in the death of the animal. Conversely, overfed larvae may molt twice in rapid succession without undue increase in size.

Analysis of Segment Formation

Observations and definitions

The larval development of Artemia can best be dealt with in terms of the number of body segments present. The first three segments become visible almost simultaneously at a total larval size of 0.745 mm. (stage 3), after the embryonic yolk has been digested away, and the termination of the hatching, nauplius, and metanauplius stages can therefore be represented as the termination of stages 0, 1, and 2, respectively; at the end of any following stage the stage number will thus indicate directly the number of body segments present. It will be convenient to distinguish between a thoracic period of development, comprising the first 11 stages, and an abdominal period, including stages 12 to 19; the latter can again be divided into a genital period (stages 12 and 13) and a post-abdominal one (stages 14 to 19).

Except for the first three, each individual segment is initially recognizable as a transverse ring of thickened mesoderm, the segment rudiment, immediately underneath the otherwise smooth epidermal layers (segmental stage *a*). Later, partial transverse constrictions appear externally in the epidermis and the chitin, in a plane just posterior to that of the segment rudiment (segmental stage *b*). Eventually, the constrictions become complete and deepen, with a concomitant bulging out of the body wall in the region of the segment rudiment (segmental stage *c*). At this stage, the segment can be considered "laid down," its shape resembling more or less a short cylinder. In thoracic segments, appendage buds appear in stage *c* ventro-laterally, on either side. The segments are considered mature when their pairs of swimming appendages first become independently motile. Stages *a* to *c* of the first and second, and stages *a* and *b* of the third segment can never be clearly seen; the first stages of these segments are attained prior to hatching and during the nauplius and metanauplius phases, when the presence of dense yolk conceals details of structure. As these segments become plainly visible in the third stage of the thoracic period, segment 3 is in stage *c*, but segments 1 and 2 are already correspondingly ahead, both in size and the degree of their development.

At the end of the thoracic period the 11th segment has reached stage *c* and the first five segments have become mature. The 11th segment attains maturity at the end of the abdominal phase of development (stage 19). Appendage buds similar to those on more anterior segments also develop on segments 12 and 13. But instead of developing into swimming appendages the buds on either side of both segments enlarge, and in the female fuse into a sac in stage 18, forming the left and right brood pouch; no male larvae were investigated. The remaining six segments develop similarly from segment rudiments, but no appendage buds are ever formed and stage *c* represents the first stage of maturity. At the end of the abdominal period the 19th and last segment has become mature. In the head, the ocellus becomes pigmented in stage 2 and the compound eyes in stage 4. The maxillae and maxillulae also form in stage 2. The end of stage 19 marks the time when the gnathobase and the setae have been lost entirely from the second antenna. After stage 19 an arbitrary number of non-segmental stages ensue before sexual maturity is reached.

When individuals in identical stages of development from the same or from different salinity media are compared, it is strikingly apparent that total lengths and body proportions in general fall within well-defined size-classes; the deviations from the underlying averages in no case exceed ± 3 per cent. In Table I the averages of a variety of body measurements are shown, from the 25 individuals watched throughout development, with the stage number as the basis of calculation; these values, within ± 3 per cent, are true for individuals from any of the salinities here examined. A schematic diagram of an Artemia larva indicates, in Figure 2, how the various entities have been defined. Head length is understood to include maxillar and maxillular segments. The length of a segment refers to axial and the width to its lateral extent. Total abdominal length is the length of the segmental portion, whether actually cut up into segments or not, plus the length of a terminating anal piece; the segmental portion is the pygidium of annelid forms, and in Artemia is readily distinguished from the anal piece, or urosome, by a constriction. During the abdominal period of development, the segmental abdomen contains a genital region composed of segments 12 and 13, as well as a post-abdomen (presumptive segments 14 to 19) with segmented and non-segmented portions.

From observation and from examination of the data in Table I the following facts concerning the formation of segments in relation to larval shape and size are consistently found to occur:

1. Every thoracic segment when newly formed (stage *c*) has a fixed length of 0.03 mm. and a fixed width of 0.144 mm.
2. Every time a new thoracic segment is laid down in stage *c*, preceding segments increase in length and in width.
3. Throughout the thoracic period, the segmental part of the abdomen has a constant average length of 0.249 mm.; its anterior width, being slightly smaller than the width of the newest segment, is also constant ($C = 0.142$ mm.).
4. During the thoracic period, the lateral contour-lines of the thorax are straight lines converging posteriorly; the lateral abdominal contour-lines are also straight, but generally they converge with a greater degree of taper than the thoracic contours.
5. Appendages are longer the more anterior they are; the line joining the tips of the appendages on one side of the body is more or less a straight line.
6. As the 11th segment appears in stage *c*, the 5th segment has matured and the 19th segment has appeared in stage *a*.
7. Between stage *a* and stage *c* of the thoracic segments, 4 stages of larval development intervene; an interval of more than 4 developmental stages is necessary for an abdominal segment to reach stage *c* from stage *a*.

Table I

Segmental development and larval body proportions, in millimeters

Stage Number	No. SR	No. S	SM	Tot. L	HL	T	TAL	A	GL	P	SPAL	U	TS	TSL	PS	W	WTSL	WA	WASL	WU
0	4	0		0.425	0.206		0.219									(0.142)				
1	5	1		0.524	0.209	0.030	0.285	0.215				0.070	0.030	0.030		0.144	0.144			0.080
2	6	2		0.637	0.249	0.070	0.318	0.238				0.080	0.040	0.030		0.146	0.145			0.080
3	7	3		0.745	0.289	0.122	0.334	0.241				0.093	0.050	0.030		0.150	0.136			0.085
4	8	4		0.841	0.313	0.185	0.343	0.243				0.100	0.058	0.031		0.155	0.139			0.097
5	9	5		0.934	0.339	0.242	0.353	0.244				0.109	0.065	0.028		0.162	0.138			0.105
6	10	6		1.025	0.343	0.311	0.371	0.255				0.116	0.076	0.033		0.167	0.139			0.108
7	11	7	1	1.125	0.366	0.390	0.369	0.237				0.132	0.086	0.029		0.179	0.143			0.107
8	13	8	2	1.236	0.382	0.480	0.379	0.243				0.136	0.097	0.030		0.191	0.144			0.111
9	15	9	3	1.386	0.400	0.600	0.386	0.240				0.146	0.110	0.031		0.209	0.143			0.118
10	17	10	4	1.540	0.407	0.733	0.400	0.246				0.154	0.120	0.033		0.225	0.150			0.120
11	19	11	5	1.716	0.429	0.861	0.426	0.246				0.180	0.127	0.027		0.245	0.150			0.125
12		12		1.855	0.455	0.87	0.53		0.04	0.290		0.200	0.135	0.035		0.25		0.150	0.145	0.13
13		13		2.000	0.460	0.87	0.65		0.10	0.325		0.225	0.130	0.035		0.25		0.160	0.145	0.13
14		14	6, 14	2.155	0.475	0.93	0.75		0.12	0.38	0.06	0.25	0.135	0.045	0.06	0.25		0.170	0.148	0.13
15		15	7, 15	2.400	0.520	1.05	0.83		0.14	0.45	0.16	0.24	0.145	0.050	0.08	0.35		0.185	0.150	0.13
16		16	8, 16	2.720	0.550	1.20	0.97		0.165	0.58	0.27	0.25	0.150	0.060	0.09	0.33		0.190	0.140	0.14
17		17	9, 17	3.050	0.570	1.35	1.13		0.185	0.73	0.48	0.25	0.175	0.065	0.12	0.33		0.200	0.137	0.14
18		18	10, 18																	
			12, 13	3.450	0.600	1.50	1.35		0.19	0.91	0.75	0.25	0.175	0.075	0.15	0.35		0.210	0.139	0.14
19		19	11, 19	4.000	0.650	1.70	1.65		0.23	1.13	1.08	0.27	0.180	0.095	0.18	0.37		0.230	0.144	0.14

Legend: No. SR, number of segment rudiments; No. S, number of segments present; SM, segments matured in this stage; Tot. L, total larval length; HL, head length; T, thoracic length; TAL, total abdominal length; A, length of segmental abdomen; GL, genital length; P, post-abdominal length; SPAL, length of segmented post-abdomen; U, urosomal length; TS, length of first thoracic segment; TSL, length of last thoracic segment; PS, length of a post-abdominal segment; W, width of first thoracic segment; WTSL, width of last thoracic segment WA, width of first abdominal segment; WASL, width of last abdominal segment; WU, anterior urosomal width.

8. Between stage *c* and maturity in the first 5 segments, 6 stages of development intervene; 8 stages intervene before maturity of segments 6 to 11.

9. The anterior width of the non-segmented portion of the abdomen during the abdominal phase is of a fixed and constant magnitude and identical to the constant anterior width of the abdomen during the thoracic phase; thus at the end of stage

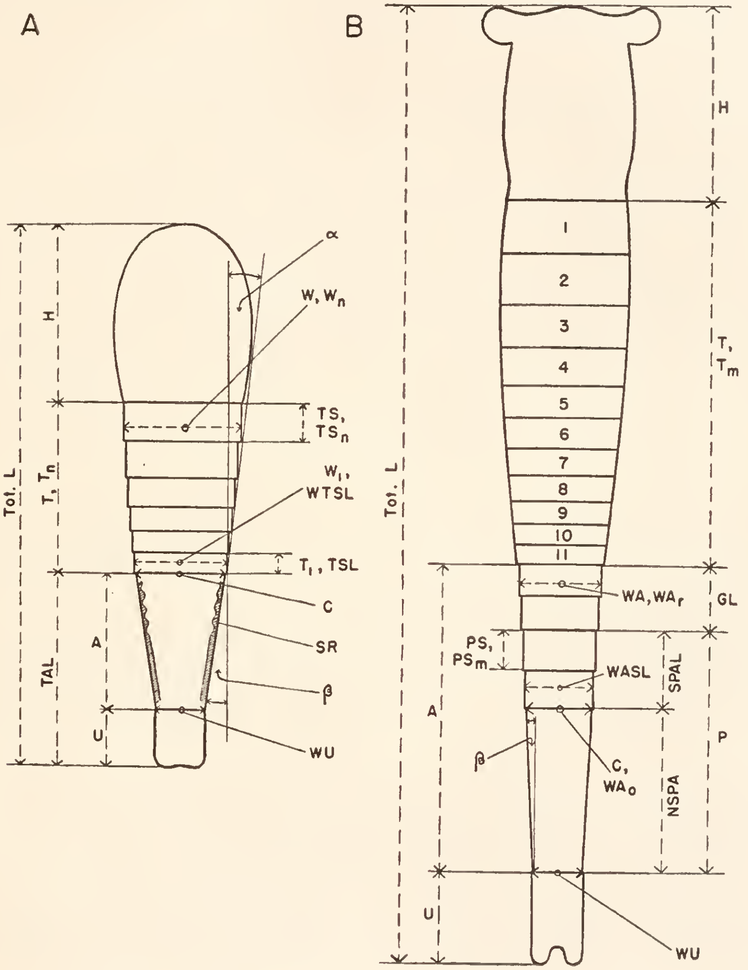

FIGURE 2. Schematic diagram of a larva of *Artemia salina*. A, larva in the thoracic period; B, larva in the post-abdominal period of development. *A*, length of segmental abdomen (pygidium); α, angle of thoracic taper; β, angle of abdominal taper; *C*, W_0, WA_0, constant anterior width of segmental abdomen; *GL*, length of genital region; *H*, head length; *NSPA*, non-segmented post-abdomen; *P*, length of post-abdomen; *PS*, PS_m, length of a post-abdominal segment; *SPAL*, length of segmented post-abdomen; *SR*, segment rudiment; *T*, T_n, T_m, length of thorax; *TAL*, total abdominal length; *TS*, TS_n, length of first thoracic segment; *TSL*, T_1, length of last (newest) thoracic segment; *Tot.L*, total larval length; *U*, urosomal length; *W*, W_n, width of first thoracic segment; *WTSL*, W_1, width of last thoracic segment; *WA*, WA_r, width of first abdominal (12th) segment; *WASL*, width of last (newest) abdominal segment; *WU*, anterior width of urosome.

19, the width of the urosome is the same as the posterior width of the head in stage 0 when segmental development started. A constant width has seemingly travelled down the larva.

10. At the beginning of stage 12, the segmental abdomen starts to grow in length at a fast rate, having retained a constant length in the thoracic period.

11. Segments 12 and 13 are of equal length at any time after their formation; they are individually always somewhat longer than the 11th thoracic segment and become progressively shorter, relatively, than the 14th segment. At stage 18, 6 developmental stages after segment 12 has reached stage *c*, segment 12 and 13 fuse to form the brood pouch in the female and can then be considered matured. The interval for attainment of maturity is thus equal to the similar interval in the first 5 thoracic segments.

12. Segments 14 to 19 are not of equal length when formed; more posterior segments, when formed, are longer than more anterior ones when formed. Also, any one of these segments has always one-sixth of the length of the post abdomen, and at a given time post-abdominal segments are of equal length.

13. When segment 19 reaches stage *c*, the 11th segment attains maturity.

14. During the abdominal phase, the thorax changes shape in the following way: the 2nd segment becomes longer and wider than the 1st, then the 3rd larger than the 2nd, etc., and the 5th becomes the largest, coincident with the end of stage 19. As a result, the lateral thoracic contours become curved, the widest part of the thorax being at segment 2 in stage 16, at segment 3 in stage 17, etc., and at segment 5 in stage 19.

15. Similar differential increases take place in the appendages; at stage 19, the 5th pair of swimming appendages is longest and appendageal length regularly decreases towards the 1st and the 11th pair. The line joining the appendage tips on one side of the thorax is now also curved.

16. During the abdominal phase, and paralleling the differential increases in the thoracic segments, a progressive dorsal thoracic curvature develops, with an analogous shift backwards of the maximal flexure; the latter arrives similarly at segment 5 at the end of stage 19. Due to this flexure the head now appears bent ventrad.

17. The lateral contours of the abdomen remain straight lines throughout the abdominal phase, with a definite taper directed backwards.

18. At the end of stage 19 segmental development is completed; further development is still to take place in the head. The essential overall shape of the animal as now established, i.e., the possession of a barrel-shaped thorax and a straight tapering abdomen, is carried through to sexual maturity, although changes of detail do still occur.

These observations are now to be interpreted and integrated analytically.

The thoracic phase of development

The lengths of the first thoracic segment, in successive stages, are 0.03, 0.04, 0.05, 0.058, 0.065, 0.076 mm., etc. (Table I). The differences between these values, taken for all 11 thoracic stages, are very close to an average difference of 0.0097 mm. The length of the first thoracic segment in successive stages can therefore be expressed as an arithmetical series

$$TS_n = (TS_1 - TS_0) + (n - 1)\cdot\Delta s \qquad (1)$$

where TS_n refers to the length of the first thoracic segment at stage n; n, to the successive stage numbers from 1 to 11 (and thus to the number of segments present at the time); $(TS_1 - TS_0)$, to the initial length of the first segment at the end of stage 1; and Δs, to the increase of segmental length per stage (0.0097 mm.). The expression would mean that the first segment grows in length by a constant amount Δs during each stage.

Since every other thoracic segment is known to start off with an identical value for $(TS_1 - TS_0)$, viz., 0.03 mm., it could be possible that other thoracic segments also increase a constant amount Δs during each stage. If that were true, then the newest segment, at any given stage, would have a length of $(TS_1 - TS_0)$, the segment immediately preceding it a length of $(TS_1 - TS_0) + \Delta s$, the third but last a length of $(TS_1 - TS_0) + 2\Delta s$, . . . etc., and the first segment again a length of $[(TS_1 - TS_0) + (n - 1)\cdot\Delta s]$. In other words the length of the entire thorax, being the sum of individual segments, should be the sum of an arithmetical series whose first term is $(TS_1 - TS_0)$ and whose last term is $[(TS_1 - TS_0) + (n - 1)\cdot\Delta s]$. This can be put as

$$T_n = n\cdot(TS_1 - TS_0) + \frac{n(n-1)}{2}\cdot\Delta s \qquad (2)$$

where T_n is the total thoracic length at stage n; and $(TS_1 - TS_0)$, the constant length of the newest segment (or the length of the first segment when in stage c). Taking Δs as 0.0097 mm. and $(TS_1 - TS_0)$ as 0.03 mm., T_n for successive values of n can be calculated. These calculated values are compared with the observed values for thoracic length in Table II; the largest discrepancy is only approximately 5 per cent, and the original suggestion is thus shown to be fact, i.e., every thoracic segment grows in length for a constant amount Δs, in each stage of the thoracic period.

A similar approach can be employed to analyze thoracic changes in width. While in stage 0 thoracic length T_n is also 0, the anterior width of the presumptive thorax is already 0.142 mm. (C). In stage 1, the width of the first segment is 0.144 mm. (Table I), and the initial increase $(W_1 - C)$, analogous to $(TS_1 - TS_0)$ in equations 1 and 2, is therefore 0.002 mm.; the anterior width of the presumptive second segment is again $C = 0.142$ mm. (each segment after stage c being regarded as a short cylinder). In succeeding stages, the width of the first segment increases 0.002, 0.004, 0.005 mm. . . . etc. (Table I). The increments per stage are then not constant, as they were for segmental length, but the figures suggest that the increases of the increments per stage might be constant. If the increment in stage 2 were 0.003 instead of 0.002 mm., the increase Δw over the initial increment $(W_1 - C)$ would be 0.001, and $(W_1 - C) + \Delta w$ would represent the increase in width during stage 2. Similarly $(W_1 - C) + 2\Delta w$ and $(W_1 - C) + 3\Delta w$ would indicate the increases during stages 3 and 4 respectively. In general,

$$(W_n - W_{n-1}) = (W_1 - C) + (n - 1)\cdot\Delta w \qquad (3)$$

would be true, where $(W_n - W_{n-1})$ represents the increase in width of the first segment during stage n. The total width increase of the first segment during the first n stages would then be the sum of an arithmetical series whose first term is $(W_1 - C)$ and whose last term is $[(W_1 - C) + (n - 1)\cdot\Delta w]$, for similar reasons as in thoracic length; or

$$W_n - C = n\cdot(W_1 - C) + \frac{n(n-1)}{2}\cdot\Delta w \tag{4}$$

and

$$W_n = C + n\cdot(W_1 - C) + \frac{n(n-1)}{2}\cdot\Delta w \tag{5}$$

TABLE II

Calculated and observed magnitudes of certain larval body regions, in millimeters

Stage No.	Thoracic length		Width of 1st thoracic segment	
	Observed	Calculated	Observed	Calculated
1	0.030	0.030	0.144	0.144
2	0.070	0.069	0.146	0.147
3	0.122	0.119	0.150	0.151
4	0.185	0.178	0.155	0.156
5	0.242	0.247	0.162	0.162
6	0.311	0.325	0.167	0.169
7	0.390	0.413	0.179	0.177
8	0.480	0.511	0.191	0.186
9	0.600	0.619	0.209	0.196
10	0.733	0.736	0.225	0.207
11	0.861	0.863	0.245	0.220

Stage No.	Length of post-abdomen		Length of a post-abdominal segment		Width of 12th segment	
	Observed	Calculated	Observed	Calculated	Observed	Calculated
12	(0.29)	(0.28)	(0.041)	(0.04)	0.150	0.147
13	(0.325)	(0.30)	(0.054)	(0.05)	0.160	0.154
14	0.38	0.36	0.06	0.06	0.170	0.163
15	0.45	0.45	0.08	0.075	0.185	0.174
16	0.58	0.57	0.09	0.095	0.190	0.187
17	0.73	0.72	0.12	0.12	0.200	0.202
18	0.91	0.90	0.15	0.15	0.210	0.219
19	1.13	1.11	0.18	0.185	0.230	0.238

Taking for $(W_1 - C)$ and Δw the values 0.002 and 0.001 mm. respectively, W_n has been calculated for successive values of n, and the comparison with the observed values is shown in Table II. The percentage discrepancies are greater than those observed for thoracic length, but nevertheless insignificant in view of the greater difficulty of taking accurate measurements of entities of so much smaller magnitude. It is to be concluded that the width of the first thoracic segment grows similarly as the length of the thorax, i.e., by adding, in each stage, another term of an arithmetical series in which consecutive terms differ by a constant amount Δw.

It must now be shown that other thoracic segments also increase in width according to equations 4 and 5; actual measurements for these segments have not been taken, but the proof can be arrived at indirectly. It is known from observation that the lateral thoracic contours are straight lines converging posteriorly. The angle of taper α (Fig. 2) is always expressed by

$$\tan \alpha = \frac{W_n - C}{2T_n} \tag{6}$$

and this angle, on calculation, is seen to be very nearly constant for successive values of n. For $n = 1$ and $n = 11$, $\tan \alpha$ equals 0.033 and 0.045 respectively; the average from all eleven values is 0.039, corresponding to an angle of 2° 18′, ± 15′. Since the contours are then straight lines, with a constant taper in all thoracic stages, the taper of individual segments must also be constant and identical, i.e., $(W_n - W_{n-1})/2TS_n$; as the length TS_n of a given segment in a given stage can be shown to be equal to the length, in the preceding stage, of the segment immediately anterior to it, an analogous equality must obtain for the width of a segment, for the taper in each case must be identical. In other words, when the width of the first segment is W_n, the width of the succeeding segment is W_{n-1}, in the same stage; this proves however, by extension, that all thoracic segments must increase in a manner identical to the first, since W_n and W_{n-1} represent sums of the same arithmetical series as that in equation 5, W_n containing one term more than W_{n-1}.

The segmental abdomen during the thoracic phase maintains a constant length ($A = 0.249$ mm.) and a constant anterior width ($C = 0.142$ mm.). The posterior width WU, identical to the "width of the urosome," however increases (Table I). The angle of taper β, therefore, expressed by

$$\tan \beta = \frac{C - WU_n}{2A}, \tag{7}$$

decreases. Stated in other words, the convergence of the abdominal contour-lines gradually diminishes. A stage will eventually be reached at which the thoracic and abdominal contours will form continuous straight lines, the thoracic contours having a constant taper (equation 6); at this time

$$\tan \alpha = \tan \beta$$

and

$$\frac{W_n - C}{2T_n} = \frac{C - WU_n}{2A} \tag{8}$$

from which WU_n can be calculated, all other terms being known. WU_n from equation (8) is 0.123 mm.; the value of WU_n closest to this in Table I is 0.125 mm. in stage 11. It follows therefore that the thoracic and abdominal contours become continuous straight lines as the end of the thoracic period of development is reached.

For analytical purposes thoracic shape during the thoracic period can be regarded as a regular cone from which the tip was cut off (frustrum of a cone). Dorso-ventral extent at any level would be very nearly equal to the lateral width at that level. The diameters of the end faces of the frustrum can thus be assumed to be W_n and W_1 respectively, and since the length of the frustrum is always given by T_n, the volume and the surface area of the thorax can be approximated by the use of known geometrical formulae. If the volume V_1 of the first segment is known the total thoracic volume V_n at any stage can also be calculated from a sum-of-a-series equation, of the general form

$$V_n = n \cdot V_1 + \frac{n(n-1)}{2} \cdot \Delta v, \tag{9}$$

which must obtain, since both length and width changes are governed by such equations. Furthermore, Δs and Δw are obviously related mathematically to Δv. In sum, if the initial size and shape of the thorax ($n = 1$), and the values Δs and Δw are known, the size and shape of the thorax at any further thoracic stage can be predicted.

The abdominal phase of development

Abdominal growth.—At the beginning of the abdominal period, the segmental abdomen starts to grow in length, having been constant before. During stages 12 and 13 the abdominal increases are 0.08 mm. per stage, or almost exactly $8 \times \Delta s$ (Table I); since the initial abdominal length at the beginning of stage 12 (or at the end of stage 11) is 0.249 mm. or approximately 8×0.03 m., it follows that during the genital period each 0.03 mm. portion of the segmental abdomen grows an amount Δs per stage. In other words, the segmental abdomen behaves as though it were already cut up into its eight segments, and each of these hypothetical segments has the same antero-posterior growth potential as thoracic segments when first laid down, viz., increasing Δs per stage after having a length of 0.03 mm. If the 12th segment were laid down in the manner in which thoracic segments are formed, it would reach stage *c* at a length of 0.03 mm. But after stage 11 the entire segmental abdomen has already started to grow, at a rate of Δs per stage per 0.03 mm. Thus at the end of stage 12 when the 12th segment reaches stage *c*, it will be $0.03 + \Delta s$, or 0.04 mm. instead of 0.03 mm. long; the entire segmental abdomen should then be eight times 0.04, or 0.32 mm., and the post-abdomen 0.28 mm. long. Analogously during stage 13, each 0.04 mm. portion of the segmental abdomen will now add an amount Δs, so that segment 13 when in stage *c* will be 0.05 mm. and the entire segmental abdomen eight times 0.05, or 0.40 mm. long. At this point the genital region should be 0.10 (2×0.05) mm. and the post-abdomen 0.30 mm. long. Actual figures in Table I support such an interpretation rather well, and the conclusion is justified that during the genital period the segmental tissue of the abdomen acquires the same growth potential in length as that of equivalent amounts of thoracic tissue during the thoracic period.

The genital region continues to grow in length at the indicated rate, as the data in Table I tend to show. The post-abdomen would similarly do so, were it not for the fact that another change in the mode of growth occurred at the end of stage 13. Successive post-abdominal lengths P_m from stage 13 on are 0.325, 0.38, 0.45, 0.58 mm. etc., in other words the increments are increasing. A sum-of-a-series expression, similar to that for thoracic width changes, fits these figures very closely, i.e.,

$$P_m - P_0 = (P_1 - P_0) \cdot m + \frac{m(m-1)}{2} \cdot \Delta p \qquad (10)$$

and

$$P_m = P_0 + (P_1 - P_0) \cdot m + \frac{m(m-1)}{2} \cdot \Delta p \qquad (11)$$

where P_m represents the total post-abdominal length for stages 14 to 19; P_0, the initial length at the end of stage 13; P_1, the length at the end of stage 14; m, the successive integers from 1 to 6; and Δp, the increments per stage over the initial increase $(P_1 - P_0)$. The theoretical value for P_0 was previously seen to be 0.30 mm., and with 0.06 and 0.03 for $(P_1 - P_0)$ and Δp respectively, the calculated values for P_m compare well with the observed ones (Table II).

If equation (11) is divided by six, the growth formula for individual segments is obtained, since each of these segments is one-sixth of the entire post-abdomen;

$$PS_m = PS_0 + (PS_1 - PS_0) \cdot m + \frac{m(m-1)}{2} \cdot \Delta(p) \qquad (12)$$

PS_0, PS_1, and $\Delta(p)$ are 0.05, 0.06 and 0.005 mm. respectively, and $(PS_1 - PS_0)$ is therefore 0.01, or very closely Δs; thus the initial increase of the presumptive segments 14 to 19, at the beginning of the post-abdominal period, is identical to the increase of these tissues during stages 12 and 13, and this increment is then augmented by a constant amount $\Delta(p)$ in each subsequent stage. What is responsible for this change in the mode of growth of post-abdominal segments? It is more than likely that non-formation of appendages is related to this, inasmuch as newly formed tissue will not be diverted for the establishment and subsequent growth of appendage buds; augmented growth of the segments would therefore be facilitated. It can now be stated in general, that while body segments are formed, length increments per stage for all segments are constant, but the increments may be added to an initial length as in thoracic and genital segments, or to an initial increase of length, as in post-abdominal segments.

As in thoracic segmentation, the anterior width of the segmental abdomen has the constant value $C = 0.142$ mm., during the abdominal period. This value is the anterior abdominal width at the end of stage 11, and the anterior width of the presumptive 13th segment at the end of stage 12. The 12th segment, by this time, has attained a width of 0.15 mm. (Table I), and in succeeding stages this width increases to 0.16, 0.17, 0.185 mm. . . . etc. As for thoracic width the increases are found not to be uniformly constant, and a sum-of-a-series expression again approaches the data best, i.e.,

$$WA_r - C = r \cdot (WA_1 - C) + \frac{r(r-1)}{2} \cdot \Delta wa \qquad (13)$$

and

$$WA_r = C + r(WA_1 - C) + \frac{r(r-1)}{2} \cdot \Delta wa \qquad (14)$$

where WA_r represents the width of the 12th segment at a stage r of the abdominal period; $(WA_1 - C)$, the initial increase in width during stage 12; Δwa, the increase in width, per stage, over the increment during the preceding stage; and r, the successive integers from 1 to 8. If for $(WA_1 - C)$ and Δwa 0.005 and 0.002 mm. respectively are taken, the calculated values for WA_r compare well with the observed ones (Table II).

Other abdominal segments can be shown to follow a similar mode of growth in width. The lateral contours being straight lines, the angle of taper β is expressed by

$$\tan \beta = \frac{WA_r - WU_r}{2A_r} \tag{15}$$

where A_r is the length of the entire segmental abdomen, i.e., genital plus post-abdominal lengths, and other values as before. Tan β, when calculated from Table I for successive values of r, centers about the average of 0.036 ± 0.004; in other words, the abdominal taper does not only remain constant during the abdominal period, but this taper is also practically identical with that reached by the segmental abdomen at the end of stage 11 (cf. above, equation 8).

Unlike thoracic segments, which start development at stage c with the same length as that of more anterior segments at stage c, the abdominal segments begin development at a length identical with that of more anterior segments at the same time. In maintaining a constant taper, the initial increase of any presumptive thoracic segment over the width C is always expressed by the first term of the series applying to thoracic width (equations 3, 4, and 5), and the later a segment arises the fewer terms of the series can it add to its width during the thoracic period. Since abdominal segments have now also been shown to maintain a constant taper, and since their lengths at stage c are equal to those of more anterior segments already beyond stage c, an analogous relation must similarly exist for segmental width; namely, the initial increase of a presumptive abdominal segment over the width C must be identical to the width increase experienced by other abdominal segments at the same time. If $(WA_1 - C)$ in equation (13) represents the initial increase of segment 12, then $(WA_2 - WA_1)$ would do similarly for segment 13. In other words, the width of both segments follow the same series, but the second term for segment 12 becomes the first term for segment 13; the third term for segment 12, similarly, becomes the first term for segment 14, etc., and the eighth and last term for segment 12 is the first and last term for segment 19. Thus as with thoracic segments, the later an abdominal segment arises the fewer terms are added to its width, but while the width increases of thoracic segments start with the same and end with consecutive terms, those of abdominal segments start with consecutive and end with the same terms of the series.

It should be observed parenthetically that equation (13) may have a slightly different constant Δwa for the genital and post-abdominal segments respectively, reflecting the different modes of growth in length of these two groups of segments; or, if the constant is actually identical the lateral abdominal contour would theoretically not be an exact continuous straight line, but rather two straight lines with slightly different taper, joined between segments 13 and 14. In either case, the difference would be so small as to be unnoticeable in practice; with the present techniques of observation and measurement, a single series relation holds for both groups of segments, and even if two separate series could be established with finer means, the principle of growth in width as outlined above would nevertheless hold.

As for segmental growth in length, a general conclusion can now be stated for growth in width, viz., width increments per stage for all body segments are constant, and the increments are always added to an initial increase in width. The combined generalization is also true, that total segmental mass increments per stage are constant, and the increments are added either to initial masses or to initial increases of mass.

Thoracic growth.—One of four possible reasons could *a priori* be advanced in an attempt to account for the differential size changes in the thorax, such that the 5th segment ultimately becomes largest, during the abdominal period: i.e., either the segment rudiments in stage *a* differ in initial size but follow the same growth curves; or the analogous converse; or either the rudiments have both equal initial size and identical growth curves; or the analogous negative. Since for all thoracic segments four stage-intervals elapse between stage *a* and stage *c*, length and width magnitudes at stage *c* are identical, and the increments per stage, no matter at which segmental stage, are identical (i.e., Δs), only the conclusion is admissible that thoracic segment rudiments have equal initial sizes and follow growth curves of the same shape. Under such conditions there are two factors which must be held responsible for the observed growth of thoracic segments, i.e., the time lag in the formation of consecutive segments, and segmental age. The time lag fully accounts for the regular gradation of segmental sizes at the end of the thoracic period and for the constant taper of the thorax; as will be demonstrated below, the influence of this original time lag carries over importantly into the abdominal phase, and this, together with the factor of segmental age, can indeed be made the basis for a consistent interpretation of the manner of thoracic growth.

Data in Table I show that thoracic length remains constant during the genital phase. Hereafter the values for length fit the equation

$$T_m = T_{m-1} + (T_1 - T_0)_m - \frac{m(m-1)}{2}\Delta s \tag{16}$$

where T_0 and T_1 represent thoracic length at the end of stage 11 and stage 14 respectively, and *m*, as before, the integers from 1 to 6. With 0.86 and 0.92 mm. for T_0 and T_1, and Δs as before, successive calculated values for T_m are 0.92, 1.03, 1.18, 1.36, 1.56, and 1.77 mm., significantly close to the observed data; the increases per stage are therefore 0.06, 0.11, 0.15, 0.18, 0.20, and 0.21 mm., and the differences between the increases are seen to diminish in a regular manner.

The scheme in Table III will account for such a series of increases. The figures in this table represent multiples of Δs and they show the length increase of the indicated segment during the indicated stage. Sums of figures in vertical rows, multiplied by Δs, indicate the increases of the entire thorax during the given stages, and successive sums are seen to be equal to the values for the increases per stage as calculated from equation (16). Horizontal sums, multiplied by Δs, give the total increments of any thoracic segment during the post-abdominal period. This scheme is reproduced somewhat differently in Table IV, in which the figures, multiplied by Δs, indicate directly the size of any of the 19 body segments at any of the 19 developmental stages; vertical sums have meanings analogous to equivalent sums in Table III.

It will be observed that all formulae previously deduced in connection with length increases are inherent in the figures in Table IV; observational data are also incorporated. For example, the first segment when reaching maturity in stage 7 has a length of 0.09 mm. (cf. Table I). Succeeding thoracic segments must also mature at this size, in consequence to the equality of their growth curves; thus segment 5 is shown to mature in stage 11 when the 19th segment appears in stage *a,* and segment 11 in stage 19, in conformity to the observational data in Table I. The scheme in Table IV also shows well the successive segmental proportions in the thorax during the abdominal phase. In stage 16, segments 1 and 2 are longest, in stage 17 similarly segments 2 and 3, etc.; maximal segmental length thus shifts caudad, fully corroborating observation.

Segmental growth of the thorax as indicated in the table can be interpreted provided two assumptions are postulated, i.e., (*a*) a segment can no longer grow by regularly increasing amounts after having passed through 14 segmental stages, counted from stage *c,* and (*b*) a segment, in order to grow by increasing amounts at all, must have matured within the first 6 segmental stages of its existence, counted from stage *c.* These two provisions constitute the limiting conditions of segmental age.

TABLE III

Scheme of segmental increments, in multiples of Δs, in the thorax during the post-abdominal phase of development

	Stage						Total segmental increases
	14	15	16	17	18	19	
Segment							
1	0	1	1	1	1	1	5
2	0	1	2	2	2	2	9
3	0	1	2	3	3	3	12
4	0	1	2	3	4	4	14
5	0	1	2	3	4	5	15
6	1	1	1	1	1	1	6
7	1	1	1	1	1	1	6
8	1	1	1	1	1	1	6
9	1	1	1	1	1	1	6
10	1	1	1	1	1	1	6
11	1	1	1	1	1	1	6
Total thoracic increases	6	11	15	18	20	21	

Table IV reveals that only the first 5 segments fulfill the second condition; segments 6 to 11 would also have matured in 6 stages of their individual existence, were it not for the fact that no thoracic growth takes place during the genital period, and maturation of the posterior thoracic segments is therefore delayed by two stages. Thus only the first five segments would be able to grow by increasing amounts, whenever such growth was made possible. It has been shown previously that at the beginning of the post-abdominal phase, the post-abdomen ceases to grow by constant increments and begins growth by increasing increments, with an initial increase during stage 14 equal to that of stage 13. Apparently the phenomenon of increasing increments at this time is not confined to the post-abdomen but also affects thoracic segments, subject to the limiting provisions stated above. Thus the first five segments have an initial increase equal to the increment during stage 13, viz., 0; segments 6 to 11, not fulfilling condition (*b*), simply continue at their former constant rates, viz., Δs per stage (cf. data in Table III, under increases during stage 14). From here on, the first five segments augment their increases by Δs in every stage, until their 14th segmental stage is passed; then, by assumption, the increment of the 14th segmental stage can no longer be augmented, but is retained as a constant increment till growth stops altogether. Thus segment one has increased its increment of zero by Δs at the end of stage 15 (Tables III and IV); but at this point its 14th segmental stage has already been passed and hereafter only a constant increment of Δs per stage is possible. Segment 2 on the other hand is younger than segment one, being laid down in stage *c* with a time lag of one developmental stage. By the end of stage 15, therefore, when segment one has just passed its 14th segmental stage, segment 2 has only passed its 13th segmental stage and its increment of Δs during stage 15 can be augmented once more by Δs; when the 2nd segment has passed its 14th segmental stage, its in-

TABLE IV

Scheme of growth of body segments, in multiples of Δs
(a refers to segmental stage a of any given segment)

	Stage																		
	Thoracic phase											Genital phase		Post-abdominal phase					
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
Segment																			
1	3	4	5	6	7	8	9	10	11	12	13			13	14	15	16	17	18
2		3	4	5	6	7	8	9	10	11	12			12	13	15	17	19	21
3			3	4	5	6	7	8	9	10	11			11	12	14	17	20	23
4				3	4	5	6	7	8	9	10			10	11	13	16	20	24
5	*a*				3	4	5	6	7	8	9			9	10	12	15	19	24
6		*a*				3	4	5	6	7	8			9	10	11	12	13	14
7			*a*				3	4	5	6	7			8	9	10	11	12	13
8				*a*				3	4	5	6			7	8	9	10	11	12
9					*a*				3	4	5			6	7	8	9	10	11
10						*a*				3	4			5	6	7	8	9	10
11							*a*				3			4	5	6	7	8	9
12							(*a*)					4	5	6	7	8	9	10	11
13								*a*					5	6	7	8	9	10	11
14								(*a*)						6	7.5	9.5	12	15	18.5
15									*a*						7.5	9.5	12	15	18.5
16									(*a*)							9.5	12	15	18.5
17										*a*							12	15	18.5
18										(*a*)								15	18.5
19											*a*								18.5

creases in subsequent stages will therefore be 2 Δs per stage. Similarly, segments 3, 4, and 5, each being one stage younger than the preceding segment, are able to augment their increments by Δs 3, 4, and 5 times respectively, before they complete the 14th segmental stage. Segment 5 in consequence is as long as segment 4 at the end of stage 19, but the former will continue to grow at a rate of 5 Δs per stage, while the rate of the latter can only be 4 Δs per stage; at any time after stage 19 therefore the fifth segment will be longest.

Analogous changes occur with regard to thoracic growth in width, and the thoracic cone-frustrum of stage 11 gradually assumes the shape of a barrel, with the "waist" at segment 5 after stage 19. The dorsal thoracic curvature of the animal, arising similarly after stage 11, can also be interpreted as a result of differential segmental increases in a dorsal direction, according to a scheme resembling that in Table III.

The genital segments have been noted to mature, i.e., to form a broodpouch, in stage 18. Table IV reveals that at the end of this stage, segment 12 has just completed its 6th, and segment 13, its 5th stage of segmental development, counted from stage *c*. Thus both segments fulfill one of the two age conditions assumed for thoracic segments; the fulfillment of the other might be expected. Observation proves that this is actually so. Genital segments of older larvae are known to bulge considerably beyond the general abdominal contour, giving them a knobby appearance. This could not be possible if the constant increases observed up to stage 18 were maintained any further; rather, after stage 18 the initial increment of an increasing rate will again be equal to the increase during the stage just passed, viz., Δs, and during a 20th stage this increment will be augmented by a given amount, during a 21st stage by twice this amount, etc., till the 14th segmental stage is passed.

The post-abdominal segments have previously been shown to grow by regularly augmented increases as soon as they are laid down. But since these segments bear no appendages, stage *c* for them is equivalent to attainment of maturity, as already observed above. Maturity thus proves to be an important temporal threshold for all body segments, and the statement that augmented growth will occur in any mature segment, provided maturity was reached in a definite time, has general application; the concept of segmental maturity is apparently not only a working hypothesis, as has been assumed at the start, but seems to have real biological meaning.

There is no doubt that the scheme of growth here presented describes correctly the actual events of later thoracic development; but the assumptions, while justified by the interpretations they allow, still remain to be explained. Only tissue culture studies will be able to reveal why segments not matured in the first 6 stages of existence are at too early a stage of development, and why segments after 14 stages of existence are at too advanced a stage to do more than keep up a constant rate.

Growth of appendages; integration of segmental development

In the preceding section it has been reasoned that segment rudiments in the thorax have equal initial size and identical growth curves; observation tends to confirm not only this but also that equal-sized rudiments develop for all body segments. It can be assumed that in these rudiments certain tissue masses (appendage rudiments), initially also of equal size and of equal growth capacity in equal times, differentiate independently towards the establishment of appendage buds. Such buds however never appear in the post-abdomen, and when they appear in other regions they may develop into swimming appendages or into a broodpouch. In the evidence presented in Tables I and IV, an important clue can be found to at least one of the factors preventing serial analogy despite the observed serial homology in appendageal development.

Every thoracic appendage rudiment reaches the bud stage after an interval of four developmental stages. The segment as a whole is at stage *c* at this point, and the appendage buds of any thoracic segment must be of identical size, due to the identity of initial size and of growth capacity for all appendage rudiments, and of identical shape, since every thoracic segment at stage *c* has identical proportions. Enough appendageal tissue has apparently been manufactured, during the four preceding stages, to initiate the development of a swimming appendage.

When a genital segment reaches stage *c*, 4 + and 5 developmental stages have elapsed since stage *a*. The appendage rudiments therefore have time to manufacture proportionately more appendageal tissue, at the same intensity as that of thoracic rudiments. If the genital segments in stage *c* had larger sizes, proportionate to the longer time interval available, the appendage buds of genital segments would have the same size and shape as those of thoracic segments. However, both the length and the width of genital segments are greater in stage *c* than the size which would be proportionate to the longer time of formation. The length of any thoracic segment when laid down is 0.03 mm., for example, and four developmental stages have elapsed since stage *a*; the length/time ratio is thus 0.03/4. In genital segments this ratio is larger, viz., 0.04/4 + and 0.05/5, and analogously for width. Appendageal tissue in genital segments can therefore not be developed in sufficient quantity, in proportion to segmental size, to produce appendage buds of dimensions equal to those of thoracic buds, even though more time is available. Genital buds will thus be relatively smaller and flatter, and the amount of appendageal tissue manufactured will be spread more thinly over the presumptive appendage region; the quantity of tissue present per unit area is apparently already below the threshold necessary for the formation of comparatively specialized swimming appendages, and only enough tissue is available to initiate the formation of a relatively simple sac.

In post-abdominal segments at stage *c* the size/time ratio becomes progressively larger still, and appendageal tissue consequently cannot even accumulate in quantities sufficient to form a bud.

After the appendage buds are laid down, an appendage retains a definite size-proportionality to the segment bearing it. When, for example, the thoracic contour is a straight line, during the thoracic phase of development, the line joining the tips of the appendages on one side is also a straight line, and, as with the segments themselves, the time lag in bud formation accounts for the taper. Similarly, as the thorax gradually becomes barrel-shaped in the abdominal phase, the transformation is reflected in differential length increases in the appendages, and when the appendageal tips on one side of the body are joined by a line, the result is an analogously barrel-shaped contour.

From the above analyses, the following integrated sequence of events becomes apparent with regard to segmental development.

Shortly before hatching segmental rudiments of equal size begin to be formed, at a rate of one per developmental stage; with a time lag of four stages, segments are constricted off in posterior succession, all with constant initial sizes and increasing by a constant amount during each stage. As the first segment reaches maturity, the rate of segment rudiment formation increases to two per stage. Rudiments are laid down at this rate till the newest rudiment appears at the posterior end of the segmental abdomen which latter had so far maintained a constant length. The last formed rudiment happens to be the 19th and by this time, 11 segments have been constricted off, five of which have already matured.

The process of rudiment deposition and segment constriction could be assumed to go on at length, were it not for the fact that the "end" of the animal has been reached. This is apparently the cue for a general change in the mode of growth. The entire segmental abdomen begins growth, increasing as yet equal amounts per stage, and the thorax ceases to grow. After two genital segments of equal size are formed another general change occurs, to the effect that hereafter any segment maturing within a definite time may grow by augmented increases, as described in detail above. This type of growth is maintained, in each segment in which it takes place until the 14th segmental stage is passed, whereupon the total increment of the 14th stage is reproduced without further increase in each succeeding stage. Segments not matured within the required time continue to grow by constant increments. The eventual result of this varied manner of growth, maintained up to sexual maturity, is the barrel-shape of the thorax, the presence of a dorsal thoracic curvature, the knobby appearance of the broodpouch segments, etc.

Discussion

Throughout the present analysis of metamerism in *Artemia salina,* the time scale employed was that of developmental stages, defined as the number of body segments present. It must be eminently realized that this is a scale of relative, biological time. Events in nature take place in a space-time continuum, and to Artemia equivalent happenings in space, i.e., the establishment of segments, must be correlated to the passage of equivalent units of (relative) time, i.e., what here had been called "stages." In hours and minutes, segment formation occurs of course not in equivalent times, since the phenomenon is dependent on the environment on the one hand, and on changes in growth rates with age on the other. Artemia and other similarly primitive forms are particularly suited for a ready identification of relative time, but in segmented animals of greater complexity, as well as in non-segmented groups, "equivalent happenings in space" cannot be picked out with comparative ease, and it will be more difficult to tell what the relative time scale actually is; but that it is intrinsically present in biological phenomena has already been acknowledged by others. Thus Needham (1942), after briefly reviewing the pertinent literature, states:

> "Mouse time must bear the same, or a similar, relation to elephant time as mouse spatial magnitudes to elephant spatial magnitudes. Indeed, unless the time factor is brought into account, we may understand morphological similarity, but we can never hope to understand physiological, still less embryological, similarity."

Measurements on Artemia in absolute time would never have brought to light the truly amazing simplicity of the laws of segment formation, as given by the series and the sum-of-series formulae, and in terms of relative time these formulae assume a simple biological meaning, viz., (*a*) that equivalent spatial events take place during equivalent relative times, and also (*b*) that equivalent spatial events take place in tissues of equivalent relative age. For illustration, the thorax during the thoracic period of development may be considered, where the increments per stage of $(TS_1 - TS_0)$ and $(W_1 - C)$ (equations 1 and 3) are indeed equivalent and constant, and where every other segment grows similarly in this same manner; summation of the increments must then result in the sum-of-series expression. Analogous interpretations, based on the idea of spatial and temporal equivalence can be adduced in every other case in which the formulae hold, i.e., virtually for the entire period of segmental development. Before and after this period, relative time is of course still operative, but its expression is latent, inasmuch as its passage is not paralleled by morphological events clearly identified as equivalent. The same would be true for the majority of living organisms, but it can be asserted with a fair amount of logical conviction, that if and when it will be possible to make explicit the relative time scales of living organisms as a whole, size increments in relative time units will be found to be equivalent, and series formulae of linear, quadratic, and perhaps even of higher degree will be found to hold.

It should in general be useful to have a specific term to distinguish relative biological time from absolute duration; the concept as a whole might be called "biochronism," and the relative time scale could be said to have one "biochron" as its unit. Also, in order to transcend the usual connotations of "growth rate," "biochronal rate" could be substituted. Whenever in the text above "increase per developmental stage" was mentioned, "increase per biochron" was really implied. In this connection, the type of analysis in the present report is clearly different from "allometric," "heterauxetic," or "heterogonic" inquiries. The term "morphometry" is suggested to indicate generally any quantitative appreciation of organic size, shape, and time as an integrated dynamic pattern. While it is realized that apologies are in order, more or less categorically, for the introduction of any new term into present-day biology, it should be kept in mind that new terms become unavoidable as different methods of inquiry and fresh fields of study appear.

Two immediate issues have not been touched on at all in the present analysis. First, what determines the changes in the mode of segmental growth at the end of both the thoracic and the genital periods? That the changes occur is fairly definitely established, and this would support the view that division of segmental development into periods is real, i.e., physiological as well as morphological. But beyond that, speculation into the nature and history of the changes would be futile, for lack of direct evidence. Secondly, and this is the fundamental question in the study of metamerism, why are segments formed at all? It will readily be admitted that even an attempt to answer this problem can only be made after a great deal more is known about segmentation phenomena as a whole.

Excepting these two questions however, the final size and shape of Artemia nevertheless has here been accounted for in terms of initial body proportions much as Berrill (1941) has done for the ascidian, Botryllus. When copepods, crayfish, and other diverse crustacean forms of higher evolutionary rank are considered, similarly in possession of a barrel-shaped thorax and a straight tapering abdomen, it is perhaps justifiable to reflect that Crustacea as a group might have evolved with a single and basic geometrical pattern of growth.

SUMMARY

1. Growth and the dynamic pattern of segment formation in excysted larvae of *Artemia salina* have been quantitatively studied. The final shape of Artemia at sexual maturity can be accounted for in terms of initial shape at hatching.

2. In analyzing the pattern of metamerism, the stages of development are gauged by the number of body segments present. Growth during the entire period of segment formation is found to be governed by arithmetical series and sum-of-series relations, implying that growth increments per stage over either initial sizes or initial increases are constant and identical for thoracic, genital, and abdominal segments, respectively. Later transformations of larval shape, resulting in the barrel-shape of the thorax, the presence of a dorsal thoracic curvature, the knobby appearance of the genital segments, and the presence of a straight tapering abdomen, are accounted for analytically on the basis of concepts concerning the age of segments and the time lag involved in segment formation.

3. The presence, absence, and the difference of structure of appendages are shown to be determined, at least in part, by the size of segments when first laid down, and by the time available for appendage rudiments to form appendageal tissues..

4. The time scale employed in the analysis of the segmentation pattern in Artemia is interpreted to be a relative, biological one, and the meaning of the series formulae with regard to this relative scale is illustrated. The notion of "biochronism" is introduced, as a general concept applying to biological events in relative time.

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ELECTRON MICROSCOPE OBSERVATIONS OF THE TRICHOCYSTS AND CILIA OF PARAMECIUM

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In previous publications, electron micrographs have been shown of trichocysts (Jakus, 1945) and of cilia (Schmitt, Hall, and Jakus, 1943). Recently we have re-examined both these organelles using the shadow-casting technique of Williams and Wyckoff (1945). The new technique shows structural detail with improved clarity and reveals some features not previously visible in specimens prepared in the conventional manner.

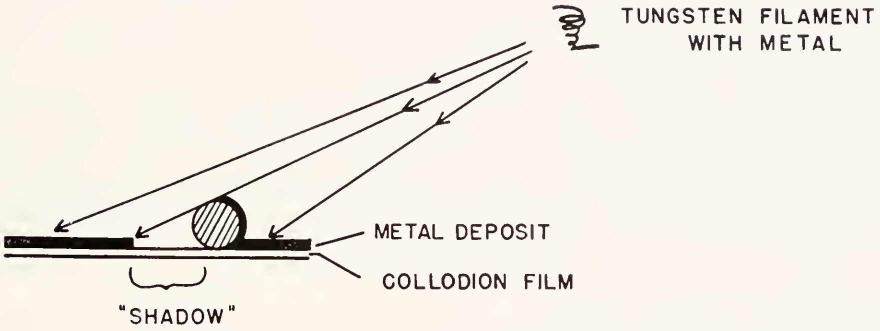


FIGURE 1. Diagram of shadow-casting technique.

The shadow-casting technique is illustrated diagrammatically in Figure 1. A specimen is placed in a vacuum bell-jar containing a conical tungsten filament in which are placed some small pieces of a suitable metal such as chromium. When the filament is raised to a high temperature by the passage of an electric current the metal evaporates, travelling in straight lines and depositing on the specimen as indicated. Structures projecting above the surface of the supporting film cast permanent shadows to the "leeward" and intercept metal to the "windward." Specimens are then examined in the electron microscope in the usual manner. In positive prints the shadows appear bright because they represent relatively transparent regions in the object. It is customary, therefore, to prepare micrographs as negative prints so that the shadows will appear darker than the background.

TRICHOCYSTS

The structure and properties of the trichocysts of Paramecium have been described in a previous paper (Jakus, 1945). In electron micrographs, the discharged trichocyst consists of a sharply-pointed tip and an elongated, cross-striated shaft with a periodicity of about 550 A. The cross-striated structure appears to be a thin membrane formed by the lateral aggregation of fine fibrils. The tip, in contrast to the shaft, is quite opaque. The reason for this opacity was not obvious.

Further information about the morphology of the dried extruded trichocyst is obtained from electron micrographs of shadowed specimens (Fig. 2). The tip is seen to be a compact structure which stands up from the film and is not flattened to any great extent as a result of dehydration. The contour of its shadow indicates that it is shaped somewhat like a golf tee. In contrast to the tip, the dried shaft is very flat, as is evident from the short shadow it casts. The cross striations previously observed are enhanced by the metal, indicating that the surface has a regularly corrugated contour. The elevated regions correspond to the darker bands in both untreated trichocysts and those stained with phosphotungstic acid. Other details of structure observed previously may also be found in some shadowed trichocysts. These are the fine longitudinal striations of the shaft membrane and the larger periodicity (2,200 A) frequently noted along the shaft. The latter may appear simply as a slight further intensification of every fourth dark band, suggesting that these ridges have a somewhat higher elevation than do the others.

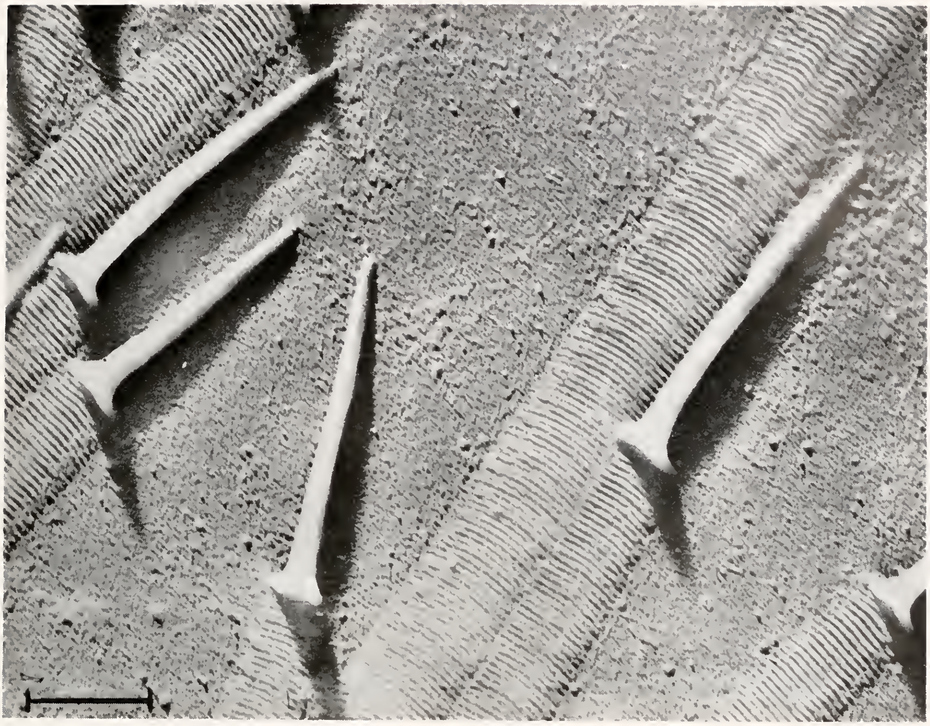
In some specimens the pointed tip appears regularly cross-striated, if the amount of metal deposited has not been excessive and the orientation of the tip is approximately parallel to the direction of deposition. This banding has not been seen in either stained or unstained specimens and, while it is readily visible in the original micrographs of shadowed tips, it is not considered to be of sufficient clarity for reproduction. Although relatively constant in any one tip, the spacing varied from 280 to 365 A in the different tips measured and had an average value of about 300 A. This is to be compared with the average period of about 550 A in the trichocyst shaft.

CILIA

The cilia of Paramecium are shed quite readily if the cell is injured and both intact cilia and fragments are observed frequently in preparations of trichocysts. Each cilium consists of a bundle of fibrils (about eleven in number), extending the full length of the cilium (Fig. 3). The diameter of the dried fibrils lies between 300 and 500 A. It may be of significance that both the number of fibrils and their diameter are in close agreement with the corresponding values observed in the sperm tails of numerous animal forms (Schmitt, Hall, and Jakus, 1943).

In fixed preparations (for example, with OsO_4), the component fibrils usually adhere to form a compact bundle, while in unfixed cilia they separate to a greater or lesser extent. They are clearly defined in shadowed specimens. Usually the separation of fibrils is not complete and they remain in close contact near the end of the cilium which was attached to the cell. Here they appear sometimes to be joined into two closely adjacent bundles.

It is not evident what holds the fibrils together in the living cilium. No spiral sheath similar to that observed in mammalian sperm tails (Schmitt, Hall, and Jakus, 1943) or in Euglena flagella (Brown, 1945) has been seen. If a sheath does exist, it must be very fragile and easily ruptured. In some cilia, a rather poorly-defined cross-striation has been noted, particularly in two or more adjacent fibrils. This striation appears to be unlike that of clearly cross-striated proteins and, if it is not an inherent periodicity in the fibril, it may represent the remnants of some binding or enveloping structure.



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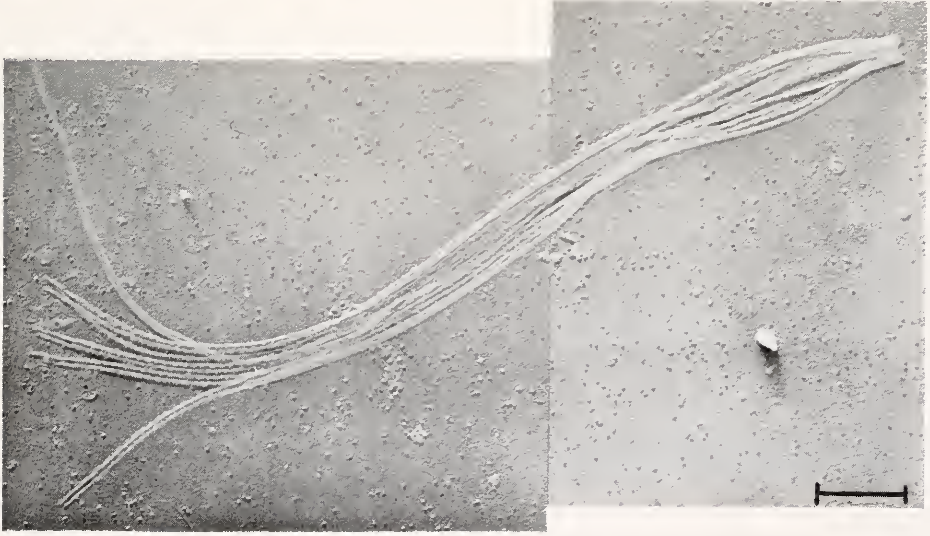

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FIGURE 2. Trichocysts from Paramecium, shadow-cast with chromium. × 16,000.
FIGURE 3. Cilium from Paramecium, shadow-cast with chromium. × 11,000.

SUMMARY

Electron micrographs of shadow-cast trichocysts of Paramecium show that the dried trichocyst shaft is flattened on the supporting film, while the pointed tip is apparently more resistant to collapse on dehydration. Accentuation, by the metal, of the cross striation previously observed in the shaft indicates that the periodicity is accompanied by corrugation of the dried surface. A cross striation in the tip is also visible in some micrographs of shadow-cast specimens. In the few cases where the periodicity could be measured, the average spacing was about 300 A, as compared to about 550 A for the well-defined shaft striation.

In electron micrographs of shadow-cast specimens of Paramecium cilia, the component fibrils are seen with greatly increased clarity.

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HYDROSTATIC PRESSURE EFFECTS UPON THE SPINDLE FIGURE AND CHROMOSOME MOVEMENT. II. EXPERIMENTS ON THE MEIOTIC DIVISIONS OF TRADESCANTIA POLLEN MOTHER CELLS

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INTRODUCTION

Hydrostatic pressure increments are known to reduce progressively the rigidity of plasmagels and the viscosity of plasmasols. Eventually complete solation results. Marsland (1939 and 1942) has been able to formulate what appears to be a general quantitative law on the basis of a considerable volume of work with very diverse material. He has found that with each increment of 1,000 lbs./in.^{2} hydrostatic pressure, the relative rigidity or viscosity decreased to 76 per cent of the initial value. This applied no matter whether the cytoplasm of amoebae, *Arbacia* eggs, or *Elodea* was being studied. Furthermore, these direct effects have always proved very rapidly reversible when the pressure was released. The subsequent pattern of cell events, however, has sometimes been found to have been changed by new reorganization patterns (cf., Pease, 1940, 1941).

In the first paper of this series (Pease, 1941), experiments were reported in which advantage was taken of these known effects of hydrostatic pressure to study the first cleavage division spindle apparatus in *Urechis* eggs. The material was not well suited for this sort of work, and some interpretations were open to question. However, the following facts were clear and significant. 1) Pressure could so affect the cell that no trace of the spindle figure appeared in the fixed preparations, and presumably the spindle had been completely liquified. 2) The pressures destroying the spindle blocked all anaphase movement. 3) The chromosomes aggregated in clumps (originally thought to be vesicles) under lower pressures than were required to block anaphase movement. 4) Numerous cytasters appeared in material given a brief recovery period before fixation. 5) Peculiar "half-spindles" developed *de novo* within cytasters whenever the latter came in contact with nuclear material. 6) By their very nature, the half-spindles lacked "continuous fibers" since only one pole was involved, and also there were no "interzonal fibers." 7) Yet there was ample evidence that such half-spindles were functional in moving chromosomes, and even recently-formed nuclei with membranes were at least deformed, and probably moved, by them. The role of cytoplasmic components in the spindle was stressed (perhaps unduly), and the role of the "traction fibers" minimized (perhaps incorrectly as will be seen later).

To find out whether or not nuclear gels behaved in the same manner as cytoplasmic gels when hydrostatic pressures were applied, the extraordinary equational meiotic division in *Steatococcus* spermatocytes has been studied in unpublished work by the author. In these cells the spindle is formed inside the nuclear membrane, and the anaphase movement nearly completed, before the nuclear membrane disintegrates. In this case, there can be no question but that the whole spindle apparatus is of nuclear derivation. It was found that sufficiently high pressures destroyed it by liquefaction, and anaphase movement was blocked. The spindle re-formed once more when the pressure was removed and the cells allowed a short recovery period. Thus the physiological action of hydrostatic pressure appears to be qualitatively identical in gels of nuclear and cytoplasmic origin.

For the present work, *Tradescentia* pollen mother cells (PMC) were selected as material for several reasons. The spindle is characterized by relatively enormous "traction fibers" going to the poles from comparatively large and easily visible kinetochores. The cells have the advantage of a small number of chromosomes which are relatively large. The only important disadvantages are the impossibility of getting controls which necessarily divide at the same time as the experimental material, and the extreme difficulties (which proved insuperable with pressure techniques) of actually observing the divisions *in vivo* (cf., Shimakura, 1934).

The material was collected and prepared at Stanford University, and the author is indebted to Dr. Reed Rollins of that institution's botany department for technical advice on handling procedures and for the plants which were used. The material was studied mostly at Columbia University before the war interrupted the work. Dr. F. Schrader, Dr. S. Hughes-Schrader, and Dr. H. Ris followed its course with interest, enthusiasm, and valuable suggestions. Dr. C. W. Metz of the University of Pennsylvania also contributed excellent comments on an early draft of the manuscript.

MATERIAL AND METHODS

The half dozen *Tradescantia paludosa* plants used in these experiments possessed six pairs of chromosomes. They were derived from a common stock. The anthers were prepared by separating the connective which joins the two lobes. One lobe was then fixed as a control just at the time of pressure application to the other lobe. The bisection of the anthers with a small lance could be accomplished easily without rupturing the anther lobe walls. The lobes were handled and finally mounted in a 7.4 gm./100 ml. saccharose (Merck, C. P.) solution which Shimakura (1934) has found to be isotonic with *Tradescantia* pollen mother cells.

The pressure bomb used in these experiments held a half dram homeopathic vial, and was so designed that it could be opened very rapidly. After filling with sugar solution and a few anther lobes, the vial was sealed with "Parafilm" wax sheet held in place with a rubber band. The experimental material was always kept under the desired hydrostatic pressure for a one hour period. In a few experiments the material was fixed 30 minutes after the release of pressure which allowed time for some recovery. But in most of the experiments, the pressure was released, the bomb opened, and the fixative added within one minute. Preliminary experiments had shown that there was no appreciable reorganization within that short time limit.

Experiments were performed using 1,000 lb. pressure increments from 1,000 to 6,000 lbs./in.^{2}, and with 8,000, 10,000, and 15,000 lbs./in.^{2} Control experiments were performed giving identical treatment, but at atmospheric pressure, and at the relatively low pressure of 150 lbs./in.^{2}

Bouin's fixative, to which 3 per cent urea was added, was used throughout. For study, eight micra sections were prepared, and stained by Heidenhain's hematoxylin method. Both mordanting and staining were prolonged (never less than 5 hours each), and the sections were destained in saturated picric acid in such a fashion that considerable stain remained in the cytoplasm. There was a good deal of shrinkage, but the cytoplasmic differentiation (particularly of the spindle) was good.

Results

Effects upon the first division spindle

The first division spindle was particularly sensitive to a critical hydrostatic pressure that was found to be between 4,000 and 5,000 lbs./in.^{2} Even after 4,000 lbs. had been applied, the spindle figures looked essentially normal. There was no reduction in the length or diameter of "traction fibers" (compare Fig. 28 with Figs. 25 and 26). However, many of the "continuous fibers" had apparently been lost for the net effect was a more diffuse looking spindle mass with fewer and less conspicuous continuous fibers. The abnormalities of chromosome movement under even the lower pressures prevented any adequate study of "interzonal connections," but occasional examples that looked normal have been found after 4,000 lbs./in.^{2}

In striking contrast were the results after 5,000 lbs. had been applied. The traction fibers were then reduced in length and in diameter so that they appeared as delicate structures (Fig. 30). Small numbers of faint and very thin continuous fibers were usually visible, although not always. Ordinarily 6,000 lbs. pressure obliterated the spindle completely, but in a small fraction of the cells a fine residual fiber structure remained visible. Figure 31 is a photograph of the heaviest and most extensive fibers which have been observed in material fixed after an exposure to this pressure. It must be emphasized that this is an entirely atypical cell. No sign of continuous fibers has been seen after exposures to 8,000 lbs., and it was the very rare cell which showed indications of traction fibers. When visible, as in Figure 33 (arrows), they were thin and short. No oriented fiber structure of any sort was ever observed after exposures to 10,000 or 15,000 lbs./in.^{2}

In summary, it can be said that the first division spindle looked essentially normal after treatments with 4,000 lbs./in.^{2} pressure, but was profoundly affected by 5,000 lbs. This demarkation was really very sharp!

Effects upon the second division spindle

The spindle of the second meiotic division was considerably more resistant to hydrostatic pressure than that of the first division. The spindles appeared nearly normal after 4,000 lbs./in.^{2} pressure, and after 6,000 lbs. the spindles of some cells did not seem to be greatly affected. After 6,000 lbs. pressure there was a considerable individual variability in different cells, even within the same anther lobe. The best spindles were somewhat fainter than normal, and the fibers seemed generally thinner, but they sometimes extended from one pole to the other. After 8,000 lbs. pressure there were occasionally evidences of traction and continuous fibers, although they were always thin and faint if present. No fiber structure was ever visible after pressures of 10,000 lbs. or more.

It thus appears that the second division spindle withstood nearly 2,000 lbs./in.^{2} more pressure than the spindle of the first division. It will appear later that the pressure required to block anaphase movement was similarly proportional.

It may also be noted here that there was a little evidence that the spindles of the somatic cells in the connective were even more resistant to pressure, and were not entirely destroyed unless pressures in excess of 8,000 lbs. were applied.

Effects upon the chromosomes—fusion

Increasing hydrostatic pressures made the chromosomes progressively more "sticky" and "soft." Chromosomes tended to aggregate in fused masses. In Figure 27 a metaphase plate is shown, fixed just after the release of 2,000 lbs. pressure. It will be noted that there are stained "bridges" connecting all of the chromosomes. At this low pressure, the bridges were, on the average, only slightly heavier than comparable bridges which could be found in controls of the proper stage. However, they persisted much longer than normally, well into the anaphase stages.

When pressures of 3,000 lbs. or more were applied, the inter-chromosomal bridges tended to become much thicker, and entirely out of the range of normal variation. Figure 32 shows such connections in a cell fixed just after the release of 6,000 lbs. pressure. With progressively higher pressures, there was an increasing tendency for the fusion of chromosomes into a single mass. This can be seen in Figures 33 and 34. The extreme condition was reached at 15,000 lbs./in.^{2} when it was nearly always quite impossible to recognize individual chromosomes. This is well shown in Figure 36.

It must be emphasized that the preceding description and the photographs are typical of cells to which the pressure was applied in late metaphase stages. When the pressure was applied to early metaphases, the chromosomes showed a much greater degree of fusion for corresponding pressures. Of considerable importance must have been the proximity of chromosomes, and probably also the initial presence of thin connections. The existence of some movement in the low pressure range may have aided the process.

Not only were metaphase chromosomes fused together by treatment with hydrostatic pressures, but a comparable effect was observed with late diakinesis chromosomes before the nuclear membrane broke down. Here the chromosomes are apparently normally kept separate from one another by gel structure within the nucleus, for nucleoplasm strands showed clearly enough in fixed preparations. These strands continued to be visible until pressures of 6,000 or 8,000 lbs./in.^{2} were applied. As long as they were present the chromosomes kept apart and did not fuse. After the higher pressures the strands were no longer visible, and the chromosomes were all in a single clump together. But, as with the metaphase chromosomes, the individual chromosomes did not lose their visible identity until pressures of 15,000 lbs. were applied.

At metaphase, the chromosomes were not only found fused laterally in the plane of the equatorial plate, but the homologous chromosomes were also fused together so that their separation was greatly complicated. This was very obvious when first division anaphases fixed just after the release of 3,000 or 4,000 lbs. pressure were studied. Practically every cell showed evidences of fusion with bridges that were often long and massive (cf., Figs. 1–12). Such bridges always stained just as the chromosome proper with hematoxylin (Fig. 39), and the larger ones, at least, were stained by the Feulgen reaction. These bridges were frequently between homologous chromosomes, but also commonly involved lateral fusion with non-homologous chromosomes.

Even more massive bridges were found in the second meiotic division material subjected to the higher pressures which still allowed a good spindle to exist. Then, after 6,000 lbs./in.^{2} pressure, most or all of the chromosomes were frequently so fused together that they nearly lost their visible identity. However, the mass of chromosomes often would be strung out from one end of the cell to the other (Fig. 15).

It should be noted that the chromosomes of somatic cells showed the same type of fusion. These have occasionally been seen in the tissue of the connective, and Figure 41 shows one bridge out of a total of three present in such a cell fixed just after the release of 4,000 lbs. pressure.

Effects upon the chromosomes—rounding

It should be emphasized that all of the fusion bridges between chromosomes had rounded outlines. This shows well in Figures 27 and 32, and suggests a considerable plasticity.

In addition, the chromosomes as a whole tended to round up under the higher pressures. This was most obvious in the second division chromatids which were V-shaped with relatively long and thin arms. After 3,000 lbs. pressure there was very little noticeable change in shape even though there might be some fusion (Fig. 13). However, after 4,000 lbs. there was a striking alteration. The chromatids were then decidedly thickened and shortened (Fig. 14). This tendency became more pronounced with increased pressures (Fig. 15, 6,000 lbs.).

The short and thick chromosomes of the first meiotic division were not as suited for study, but the same tendency was obviously present. Particularly after 10,000 lbs., when the identity of individual chromosomes could still be seen, they were decidedly shortened and rounded except at the kinetochore region (Fig. 34).

Effects upon the chromosomes—the spindle attachment region

The first meiotic division material gave the impression that 1,000–3,000 lbs./in.^{2} pressure allowed a greater extension of the attachment region of the chromosomes than was normal (compare Fig. 26 with 25). More particularly, this region of some chromosomes was extended far beyond what could be found in the controls. The attachment region gave the impression of being unduly short in the material exposed to 4,000 lbs. pressure. An attempt to measure statistical samples was decided upon.

In Table I the mean extensions of the attachment regions of first division chromosomes are given for pressures up to 4,000 lbs./in.^{2} There were, of course, real difficulties in measuring such small distances, but errors should have cancelled out in the averages. While no great reliance should be placed on the absolute values, they certainly indicate the general trend.

The measurements were made with a filar micrometer. In each group, 50 measurements were made at random, excepting that only cells in anaphase were selected, and individual chromosomes that had not yet separated and left the metaphase plate were measured. The micrometer hair was moved up to a chromosome until it just touched the distal tip of the kinetochore (indicated by the arrows in Figs. 25 and 26), and a reading made. Then the hair was swung across the field, and moved back in the other direction until the hair just touched the base of the attachment stalk which was ordinarily rather well defined from the body of the chromosome by its relative translucency. Then a second reading was made. The difference measured the length of the stalk plus the width of the hair in the micrometer. The hair width was measured in the same way in relation to a fixed point, and this value was subtracted from all of the measurements. The figures were then converted to micra. The control measurements actually used for comparison were combined from data upon the control anther lobes of the 1,000 and 3,000 lb. experimental material, and a control anther which was left mounted in the bomb for one hour before fixation, but without pressure.

It is to be concluded that the mean length of the attachment stalk was definitely increased by pressures from 1,000 to 3,000 lbs./in.^{2}, and it has also been found that there is no overlap in the extreme extensions between control cells and experimental cells exposed to this pressure range. With 4,000 lbs. pressure the mean extension was significantly less than in the controls, and the greatest extensions found after this treatment did not even approach the maxima found in the controls.

TABLE I

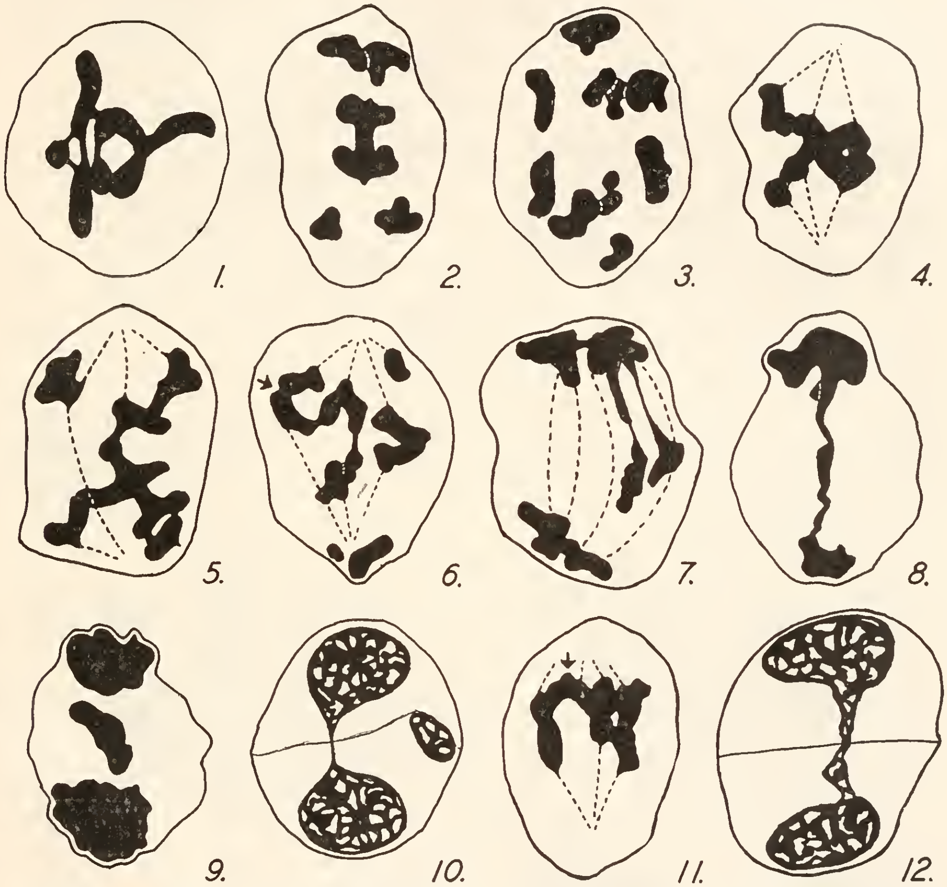
Pounds pressure	Mean extension in micra	Percentage increase in length	Percentage overlap with control mean
control	0.85		
1,000	1.4	59.4	2
2,000	1.2	38.6	6
3,000	1.2	42.9	4
4,000	0.68	−19.9	22

The distance between the tip of the kinetochore and the base of its stalk is given in the second column. In the experimental series, 50 measurements were made at random, excepting only that early anaphase cells were selected. The control average, however, is a combined average of three sets of measurements upon different material. The mean percentage increases in length are based upon figures carried to the third decimal place. The last column gives the percentage of measurements which overlapped the mean of the control.

Effects upon the chromosomes—chromonemata

We have already seen that late prophase and metaphase chromosomes fused together and rounded up under the influence of hydrostatic pressure. This, however, only applied to condensed chromosomes. Uncondensed early prophase chromosomes did not seem to be affected by even the highest pressures employed. This agrees with the findings of Pease and Regnery (1941) who were unable to detect any effect of 15,000 lbs./in.^{2} pressure upon *Drosophila* salivary chromosomes which are similarly uncondensed. It must be admitted that no detailed study has been made of the early prophase chromosomes. While there was certainly no general clumping, it is possible that very local fusions could have been overlooked, but there was no indication of shortening or thickening.

An "accidental experiment" gave further information, and additional reason for believing that the chromonemata were not affected by hydrostatic pressure. An anther lobe which had been pricked was exposed to 15,000 lbs./in.^{2} pressure for one hour and was then rapidly fixed in the usual fashion. The surrounding sugar solution had entered the anther, and apparently was somewhat hypertonic. All of the cells were slightly plasmolized and had more or less swollen chromosomes. In one small section of the anther, conditions were such that the spiral structure was visible. Figures 37*a* and *b* are photographs of one of these early anaphase cells, and it is obvious that the spiral structure was unaffected. Oddly enough there was no tendency for the chromosomes to fuse under these circumstances.

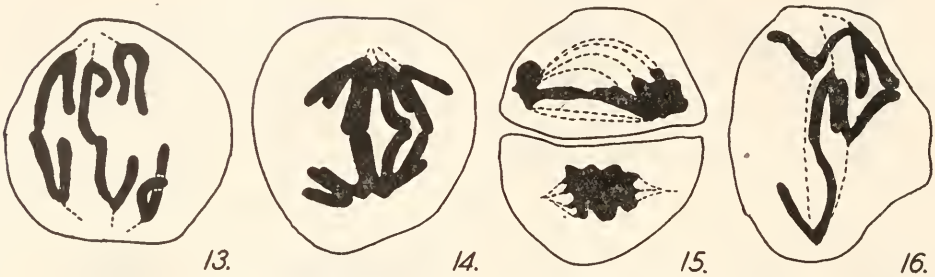


First division cells. Figures 1–10 are of sections from material which was fixed just after the release of 4,000 lbs./in.^{2} pressure. Figures 11 and 12 are of sections fixed just after the release of 3,000 lbs. pressure. The broken lines represent traction fibers except in Figure 7 where they represent the pathways of "continuous fibers." All of the chromosomes visible were not necessarily included.

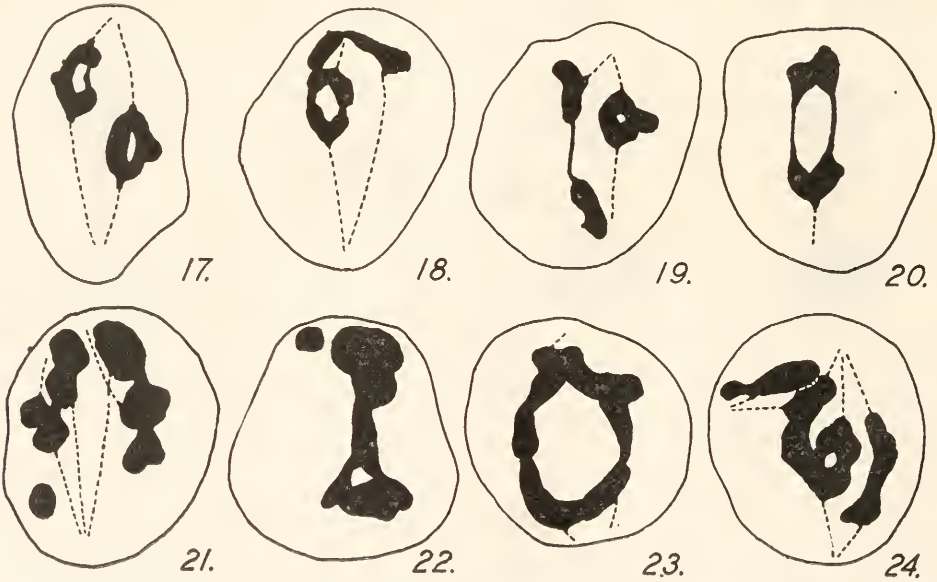
Abnormalities of chromosome movement under pressure

Because of the fusion of metaphase chromosomes, even by relatively low pressures, their ultimate distribution to the two spindle poles was usually very abnormal whenever anaphase movement took place during the pressure treatment. The particular pattern which resulted apparently depended upon the balance between anaphase forces and the local resistances of whatever fused bridges happened to be present. Greater or lesser fusions might occur between homologous chromosomes and, laterally, between non-homologous chromosomes. Almost any conceivable variation in the resulting pattern could be found in all degrees. Some of the more interesting variations which have been seen are included in Figures 1–15, which are also perfectly typical of material exposed to 3,000 or 4,000 lbs. pressure.

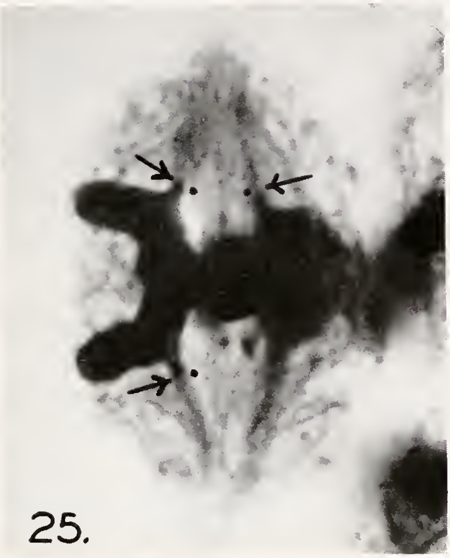
Homologous chromosomes might be so extensively fused that separation could not occur. Such pairs of chromosomes, fused as in Figure 2 in the metaphase plate

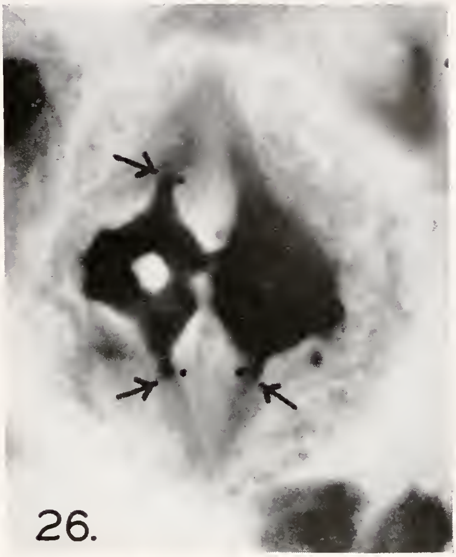


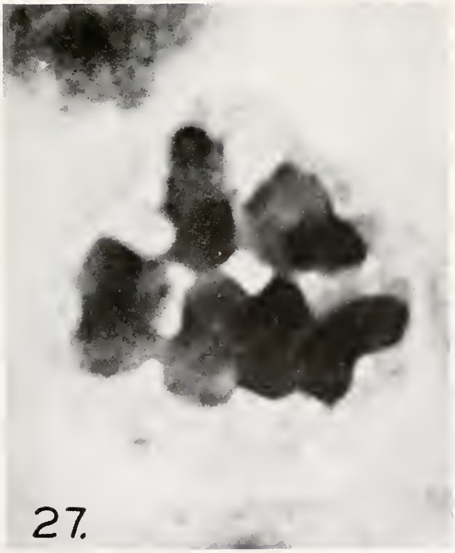
Second division cells. Figure 13 is from material fixed just after the release of 3,000 lbs./in.^{2} pressure; Figure 14, after 4,000 lbs. pressure; and Figure 15, after 6,000 lbs. pressure. Figure 16 is from recovery material, fixed 30 minutes after the release of 10,000 lbs. pressure. The broken lines indicate traction fibers except in the upper cell of Figure 15 in which they indicate the pathways of the "continuous fibers." Not all visible chromatids were necessarily included.



Figures 17–24 are all from first division recovery material which was fixed 30 minutes after the release of 10,000 lbs./in.^{2} pressure. The broken lines indicate traction fibers. Not all visible chromosomes were included except in the last three figures.







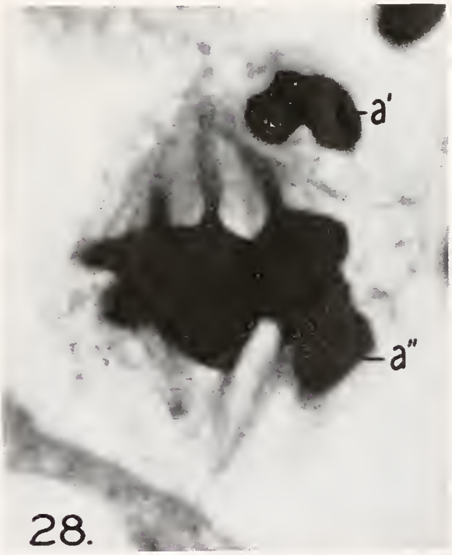


Figure 25 is a first division early anaphase control exposed in the bomb for an hour (but without pressure) before fixing. Figure 26 is of a cell fixed just after the release of 2,000 lbs./in.^{2} pressure. Figure 27 is a metaphase plate of the same material. Figure 28 is of a cell fixed just after the release of 4,000 lbs. pressure, and note the anaphase separation of the homologous chromosomes *a'* and *a''*. The small arrows indicate the distal ends of the kinetochores. The magnification of these and the following photographs is approximately × 3,000.

region, would presumably have remained there, and eventually formed micronuclei (Figs. 9 and 10).

Even though there was no lateral fusion with other chromosomes, there might be slight differences in the forces directed towards the two poles, or possibly in the strength of the traction fibers going to opposite poles. An extensively fused pair of chromosomes might then go as a unit to one pole (Fig. 5). Then there would always be an abnormally long, but otherwise normal looking traction fiber (with full thickness) going most of the way across the cell to the other pole.

Figures 4 and 5 show very extensive lateral fusion between non-homologous chromosomes. Such anaphase cells would probably have given rise to extensive bridges in telophase, and between daughter nuclei, such as are shown in Figures 8, 10, and 12.

In Figure 6 the lower member of a pair of homologous chromosomes, indicated by an arow, was laterally fused with a non-homologous chromosome going to the upper pole. Seemingly it was being carried to that pole in spite of its traction fiber to the other pole.

We have already spoken of the massive bridges which characterized the second meiotic division material exposed to 6,000 lbs. pressure, and which often involved all of the chromatids (Fig. 15). There was less fusion with lower pressures, and the abnormalities more nearly resembled what has just been described for the first division.

The critical pressure blocking anaphase movement

The best evidence for chromosome movement under pressure is certainly the presence of extensive bridging. The author sees no rational way of accounting for the bridges other than to suppose that anaphase movement occurred after the chromosomes established fusions in the metaphase plate and then pulled out the bridging connections.

With this as a criterion of movement, it is possible to state that anaphase movement continued at 4,000 lbs./in.^{2} hydrostatic pressure in the first meiotic division, but was blocked by 5,000 lbs. pressure. No extended bridge has been seen in any cell of this division exposed to 5,000 or more pounds pressure. Nor were there ever signs of asynchrony, or of directionally atypical movements.

It must also be emphasized that abnormal division resulting from fusion characterized practically *every* anaphase cell exposed to 4,000 lbs. pressure. It was also extremely common after 3,000 lb. treatments. Similar abnormalities appeared on a lesser scale after 1,000 or 2,000 lbs., but then the separation was more frequently fairly normal, and characterized only by loss of division synchrony.

In the second meiotic division very abnormal anaphase movement involving massive fusions took place in some cells exposed to 6,000 lbs./in.^{2} pressure (Fig. 15), but none was possible at 8,000 lbs.

Bridging has been found even after 8,000 lbs. pressure in the somatic cells of the connective. Figure 42 is from a somatic cell forming daughter nuclei at this pressure, and two out of a total of five bridges are visible in the plane of the photograph.

In the meiotic divisions, at least, the presence of a good visible spindle was correlated with anaphase movement. When the spindle was obviously considerably af-

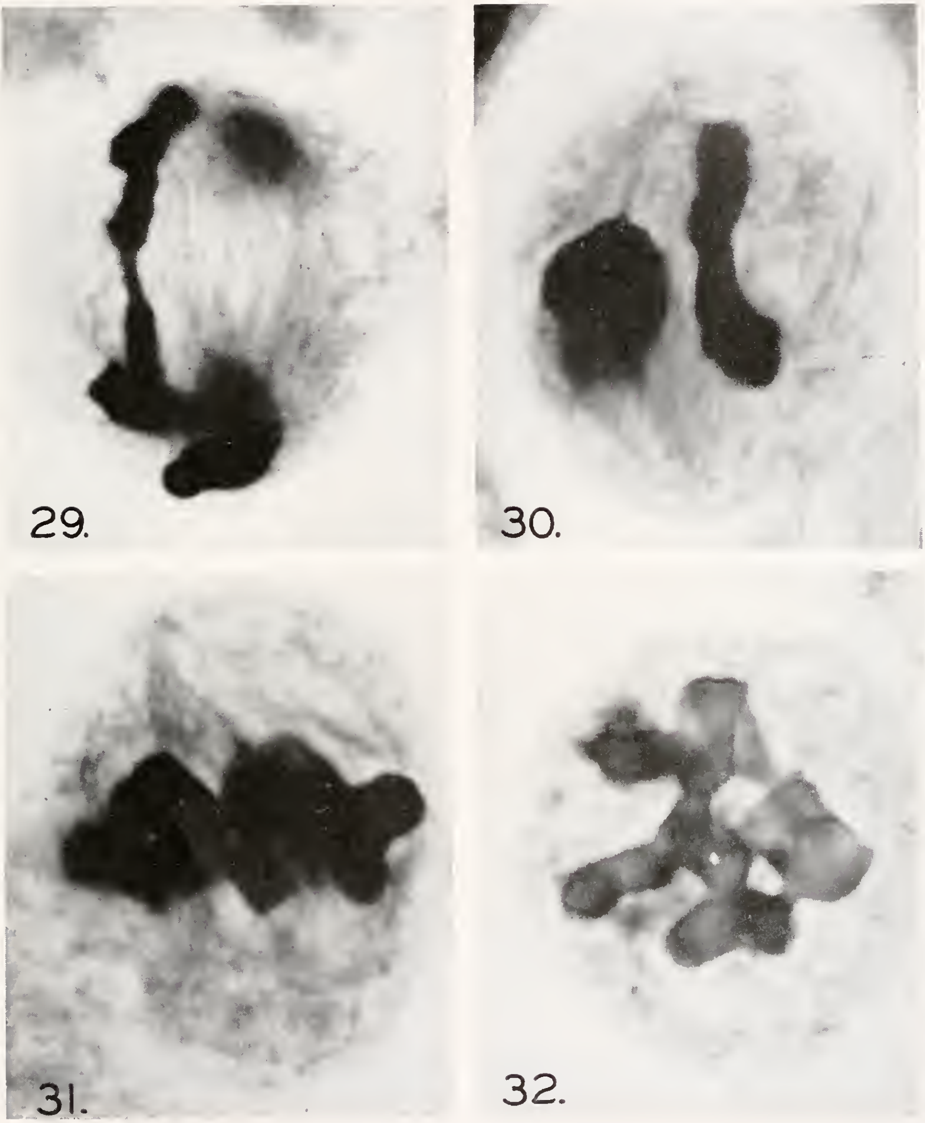


Figure 29 is a late anaphase cell from the same material as Figure 28 (exposed to 4,000 lbs./in.^{2} pressure). Figure 30 is a cell fixed just after the release of 5,000 lbs. pressure. Figures 31 and 32 are from material fixed just after the release of 6,000 lbs. pressure.

fected there were no longer evidences of anaphase movement. This was also probably true of the somatic cells, but they have not been carefully studied. It is clear that movement is most sensitive to hydrostatic pressure during the first meiotic division, withstands nearly 2,000 lbs. more pressure in the second division, and seemingly about 2,000 lbs. more in the somatic cells. This, in turn, appears due to different characteristics of the spindle gels, rather than being due to differential pressure effects upon the chromosomes. For in the first and second meiotic divisions, and probably also in the somatic divisions, the chromosomes seemed affected equally by equal pressures.

Spindle recovery after pressure release

At the time of making these experiments the importance of the recovery stages was largely unsuspected, and relatively little material was gathered. But after one hour exposures to 10,000 and 15,000 lbs./in.^{2} pressures, some experimental material was removed from the bomb and given a 30 minute recovery period before fixing. Many of these cells showed excellent spindles with massive traction fibers (Fig. 38).

Of particular interest is the fact that the traction fibers of these recovery spindles were *de novo* formations. Conclusive evidence of this was afforded by paired homologous chromosomes (still fused as a result of the pressure treatment) which formed traction fibers from both kinetochores that went to the same pole. Figure 39 is a photograph of such a condition. Figure 40*a* is a drawing of another example. Figure 40*b* seems further complicated for apparently one traction fiber had to curve around a blocking chromosome before its direction to the "wrong" pole could become definitive. In Figure 40*c* each traction fiber can probably be considered as having gone to the "wrong" pole so that the original polarity of each chromosome was entirely reversed.

Figures such as those described in the last paragraph were not rare, although out of the ordinary. They were never seen in the controls, nor is the author aware of similar accounts in the literature.

Most commonly the spindle appeared to re-form nearly along its original axis if it is assumed that the metaphase plate was not displaced, and remained as an index of that polarity. The pattern thus usually seemed very nearly normal. However, the long axis of the new spindle was sometimes very oblique to the plate, and presumably to the original spindle axis. In extreme cases a 90° shift was indicated.

Also, not infrequently multipolar spindles were found which were very rare in the control material. Three-pole spindles such as Figure 24 were fairly common, and a few four-pole spindles have been seen. All possible variants were seen with equal or very unequal poles, spaced equidistant from one another, or barely separated.

These several lines of evidence all imply that the spindle was re-formed *de novo,* and was not rebuilt upon residual structure which had survived the pressure treatment and persisted to give a framework. New patterns appeared, and whatever molecules were involved, they were at least rearranged.

The development of the recovery spindle

One can select a series of cells which apparently show the different steps of spindle re-formation after the release of pressure. In some cells fiber structure con-

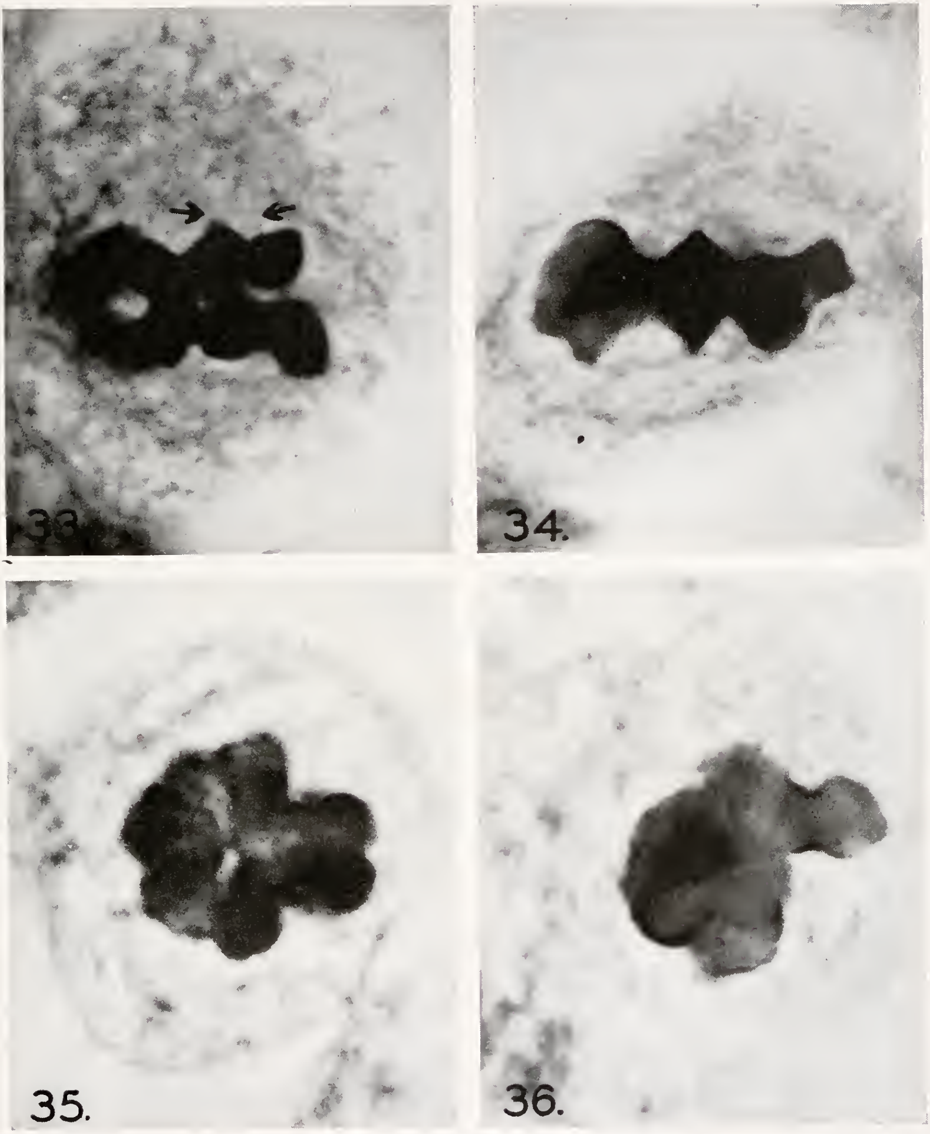


Figure 33 is of a cell fixed just after the release of 8,000 lbs./in.^{2} pressure (the arrows indicate very faintly visible traction fibers), and Figure 34 after 10,000 lbs. pressure. Figure 35 is of a cell fixed just after the release of 15,000 lbs. pressure, and the orientation is thought to be in the plane of the original spindle axis. Figure 36 is from the same material, but sectioned in the plane of the metaphase plate.

sisted of thin fibrils tangled around the clumped chromosomes of the equatorial plate, and without any polar orientation. The fiber direction was roughly circumferential to the enclosed mass of chromosomes (as in a cocoon, Fig. 44). This could be regarded as the first recovery stage.

Many cells showed polarized fibers as in Figure 45*a*. The section of Figure 45 is oblique to the spindle axis. The focus of Figure 45*a* is tangent to the slant height of the cone which makes up one-half of the entire spindle (the "surface" of the spindle, so to say). The visible fibers are the continuous fibers of the new spindle. Figure 45*b* is a lower focus of the same cell. It should be observed that there are no continuous fibers in the center of the cone. Instead, there are only slight indications of traction fibers. The continuous fibers were thus largely peripheral, but the extensive lateral fusion of the chromosomes to make a practically solid metaphase plate probably had much to do with this morphological pattern which was typical of recovery material.

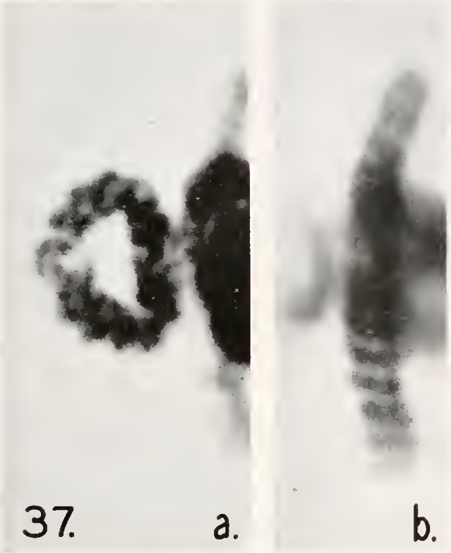
Traction fibers were not seen in cells without polarized continuous fibers. But when the latter had formed, traction fibers could usually be found. In some cells they would be thin and short, in others longer and more massive. Thus the traction fibers appeared to "grow" outward directly away from the kinetochore region, and full thickness was not achieved until they practically reached the poles.

It was possible to find many minor irregularities in the developmental pattern of traction fibers. These resulted whenever the kinetochore pointed in some other direction than directly towards a pole. A graded series could be found, the extreme examples being when kinetochores pointed more or less to "wrong" poles. Invariably the base of the traction fiber extended directly away from the kinetochore, and it did not bend towards a pole until it became associated with continuous fibers. The bend would then be towards the pole less than 90° away from the initial growth direction even if this happened to be the "wrong" pole. It thus looked as though the growth direction was unimpeded until the traction fiber became associated with continuous fibers, and then the further extension of the traction fiber followed the path of least resistance in the pattern expressed by the continuous fibers. Thus the traction fiber even developed around obstructions as in Figure 40*b*.

The fusion of traction fibers

A very rare situation casts further light on the formation of traction fibers if the interpretation is correct. It was possible to find non-homologous chromosomes in the recovery material which appeared to be bridged across the kinetochore regions. A photograph of such a bridge is shown in Figure 43. These bridges differed from all the other ordinary bridges which have been seen in that they were achromatic. Although they were short, they had exactly the appearance in the fixed and stained preparations that traction fibers had. They certainly gave the impression that they represented fused traction fibers, traction fibers which started to develop from each separate kinetochore in opposite directions, and which grew terminally into each other to fuse end to end.

The author hesitates to emphasize these structures. The material has been thoroughly searched and only two good examples have been seen, plus another which was more questionable because overlying material partially obscured it. There may be good reason for their rarity, for it is obviously an exceptional situation to have two kinetochores pointed directly towards each other. If we accept their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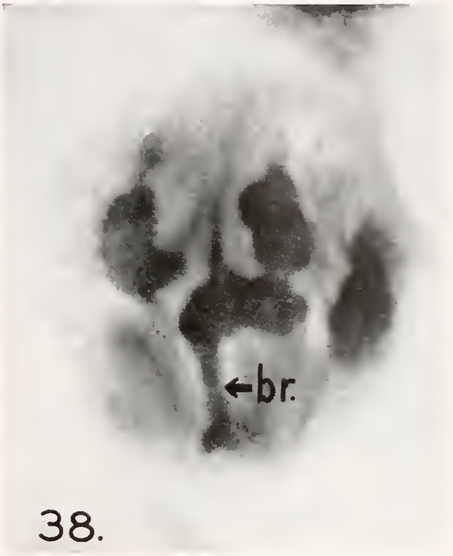
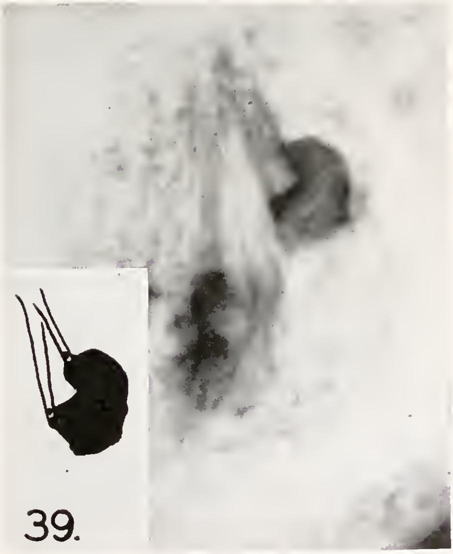

38.


39.

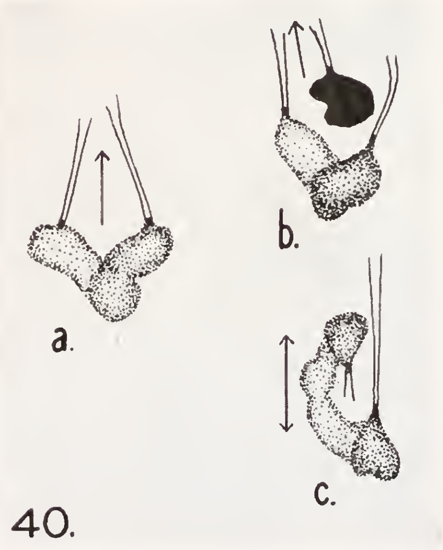
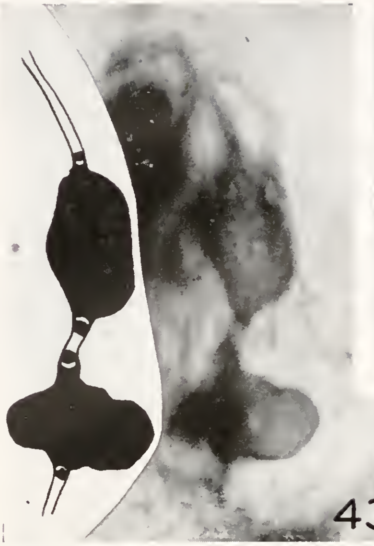
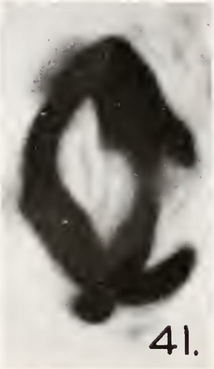

40.

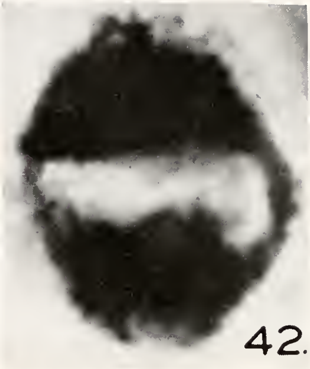
Figure 37 is from a slightly plasmolized cell fixed just after the release of 15,000 lbs./in.^{2} pressure (*a* and *b* are different focal levels). Figures 38–40 are from recovery material fixed 30 minutes after the release of 10,000 lbs. pressure. In Figure 38 note the bridge, *br.* In Figures 39 and 40 *de novo* recovery traction fibers of fused homologous chromosomes go to the "wrong" pole. The direction of a pole is indicated by arrows in Figure 40.



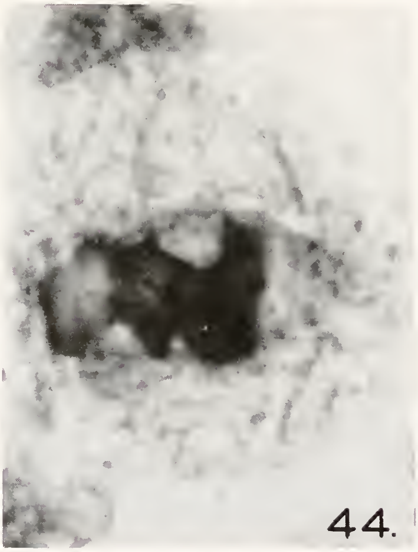
43.



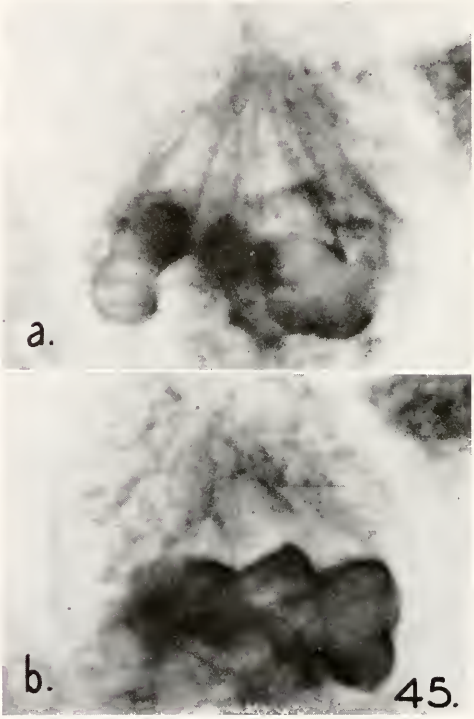
41.



42.



44.



45.

Figure 41 is of a somatic anaphase cell fixed just after the release of 4,000 lbs./in.^{2} pressure. Figure 42 is of a somatic cell forming daughter nuclei, fixed just after the release of 8,000 lbs. pressure. Figure 43 is from recovery material fixed 30 minutes after the release of 10,000 lbs. pressure, and shows achromatic bridging between non-homologous chromosomes (fused traction fibers?). Figure 44 shows an early stage of spindle recovery in material fixed 30 minutes after the release of 10,000 lbs. pressure. Figure 45 is from the same material, but spindle recovery is more advanced (*a* and *b* are different focal levels of the same cell).

reality and the above interpretation, however, the implications are of considerable interest, for it means that developing fibers can mutually terminalize each other. Yet there is no effect as far as lateral growth is concerned, and the fibers thicken as normally. There is simply no growing end left. We can say that fibers extend by terminal additions rather than from the kinetochore, or by elongation from within their length.

Having gone this far, we can make another deduction as to the role of the kinetochore in traction fiber formation. We can regard it as an "organizing center" which initiates linear extension and controls fiber diameter. The linear gowth is self perpetuating once started until the fiber reaches a pole, or is terminalized as above. The fiber thickens by further organization at the kinetochore, and additional linear growth parallel to the initially thin fiber, thus adding enclosing layers. The final fiber has a thickness equal to the diameter of the organizing center. The author reiterates that this hypothesis has a slender experimental basis, and depends upon a correct interpretation of three figures.

Chromosome movement in recovery material

There were obvious indications of chromosome movement in recovery material after the spindles re-formed. The movement was abnormal because of strong and persistent fusion bridges, and in many ways resembled the anaphase movement which occurred under low pressures (3,000 and 4,000 lbs.).

Frequently fused pairs of homologous chromosomes were found going to, or after they had reached, a single pole as in Figures 38 and 17. In such cases one traction fiber extended all the way across the cell to the other pole but seemed to be of normal thickness. This type of movement often seemed to be aided by lateral fusion with non-homologous chromosomes as in Figures 18 and 21. Less frequently the fusion between homologous chromosomes was relatively slight, and there would be a partial separation with the formation of more or less long and thin bridges (Figs. 19, 20, and 38, *br.*). Quite frequently very massive bridges were formed involving most if not all of the chromosomes which would be fused together (Figs. 22 and 23). There were no important differences between first and second meiotic division cells (note Fig. 16).

None of the material was allowed a sufficient recovery period so that daughter nuclei formed in cells which began their anaphase movement after the application of pressure. It can be presumed, however, that many of the cells would form only a single nucleus because of an inability on the part of the chromosomes to separate. Other cells would be expected to form bridged nuclei, and probably multiple micronuclei.[1]

Chromosome structure in recovery material

The persistence of chromosome fusion in the recovery material would seem to suggest just one possibility—that the initial fusion under high pressure must have been due to at least a partial liquefaction of some chromosomal element, and that the fusion bridges then gelled when the pressure was released. In the recovery material the chromosomes were thus stuck together by very viscous bridges. After examining a great deal of material, the author is of the opinion that it is very doubtful that fused chromosomes were ever able to separate completely before the formation of daughter nuclei. Most commonly there were few signs of any separation, but even in extreme cases, thin and very long bridges persisted as in Figures 19 and 20. The moderately thick bridges, at least, stained with Feulgen.

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[1] Pease (1941) definitely found this to be the case in *Urechis* eggs.

There is another, and much more puzzling, aspect of chromosome structure which is brought to light by a study of the recovery material. Even after the release of 15,000 lbs./in.^{2} pressure (which resulted in the very complete fusion of the chromosomes as in Figure 36) the chromosomes regained their visible identity and their approximately normal shape. This tendency can be seen (in 10,000 lbs. material) by comparing Figure 38 with Figure 34, but it is best seen by comparing the long chromatids of the second meiotic division (compare Fig. 16 with Figs. 14 and 15). In regaining the normal shape, the fusion areas must necessarily have been reduced in cross-section, and it is likely that some fusion bridges were lost entirely during this change. The effects of this change were best demonstrated by the separation of the second division chromatids in material recovering from 10,000 lbs. pressure. Extensive separation sometimes occurred, thus differing in degree from the first division. Figure 16 gives an indication of typical difficulties which were essentially the same as in the first division.

Absolute pressure and recovery rate

In *Urechis* egg material Pease (1941) found that the rate of recovery was roughly proportional to the absolute pressure which had been applied. In the *Tradescantia* PMC material we can only compare the effects of 10,000 and 15,000 lbs./in.^{2} pressures. Comparison is subjective, but there was not the slightest doubt but that the cells subjected to 10,000 lbs. pressure showed a much greater amount of recovery of the spindle elements in 30 minutes than the cells exposed to 15,000 lbs. showed in the same length of time. Fully developed new spindles were only rarely found in the 15,000 lb. material, but were common in the 10,000 lb. material. In both, however, the chromosomes had regained their visible identity and approximately normal shapes.

Conclusions

A single hypothesis readily accounts for most of the manifold effects of hydrostatic pressure upon spindle, chromosomes, and anaphase movement. This supposes that increasing hydrostatic pressures progressively reduce gel rigidity, with liquefaction as the end result. Conversely, after the release of pressure, conditions return to a state such that gel structures can be re-formed once more. There is, of course, an excellent experimental background for this thesis, particularly in so far as it applies to cytoplasmic systems. This has been indicated in the introduction, and has been outlined at greater length in the first paper of this series (Pease, 1941).

It is, however, unfortunate that this work depends upon an interpretation of fixed material. However, we have every reason for believing that the presence of good fiber structures in such material is a good index of oriented gel structure in life. It is only on that assumption that a comprehensive pattern appears, consistent throughout its details. It is true that whenever we have contributory evidence of liquefaction (such as a block of anaphase movement), we do not find fiber structures in the cytological material. Apparently extensive fiber structures are only precipitated by fixation agents when molecules are at least organized into an oriented pattern and probably also concentrated in a gel.

Spindle structure and formation

In view of the above considerations, it is not surprising to find that the spindle no longer appears in cytological preparations after a critical pressure has been applied before fixation. This is to be interpreted as indicating a liquefaction of preexisting gel structures, with a consequent loss of molecular organization.

It has been demonstrated that the pattern of the recovery spindle can be very different from that of the original spindle. High hydrostatic pressure seems able to break down the oriented structure of the original spindle so completely that it re-forms *de novo,* and sometimes with a new polarity. In the re-formation of the spindle much the same protoplasmic material may well be used, but the unit molecules or micells are rearranged in a different manner, just as a pile of second-hand bricks might be rearranged to build a new house. This conclusion can probably be accepted as a generalization for it agrees with the findings in *Urechis* eggs which, in their formation of "half spindles," were even more striking (Pease, 1941), with certain other observations on cytoplasmic systems (cf., Pease, 1940), and with general theory.

It is not clear just what does orient the new spindle axis in *Tradescantia* PMC. Cytasters accomplished this end in *Urechis* eggs, and obviously played the important role. These were never observed in the PMC material. Instead, we find a strong tendency for the new axis to coincide more or less with the original. The recovery spindle encountered one unusual difficulty in its organization in that the chromosomes were no longer completely separate entities. After the higher pressures there was usually a continuous plate of fused chromosomes in the equatorial region. Continuous fibers did not, indeed could not, penetrate this obstruction. However, note that homologues were not even found as half spindle components. Continuous fibers were only found sweeping around the blocking mass leaving the core of the spindle devoid of visible oriented structure except for traction fibers. Apparently, therefore, the continuous fibers are entirely a product of the cytoplasm, and are not directly related to the chromosomes. The latter, in fact, are obstacles to be by-passed. This does not, however, preclude the possibility of a generalized interaction between chromosomes and cytoplasm in that the former may "activate" the latter to form gel structures. Such an "activation" was quite definitely shown by *Urechis* eggs recovering from the effects of hydrostatic pressure (Pease, 1941). A more accurate interpretation might be not to stress the continuous fibers as such, but to consider them simply as an index of a more fundamental structural organization of molecules. They thus may signify nothing more than the basic pattern of an extensive gel framework.

On the other hand, the kinetochore apparently quite specifically "organizes" the protoplasm to form the attached traction fiber. This process is partially separable from the development of continuous fibers. We have good reason for believing that developing traction fibers simply follow the path of least resistance in the structural pattern of the bulk of the spindle, which, in turn, is expressed by the distribution of the continuous fibers. Thus the structural pattern of the body of the spindle limits the course taken by the traction fibers as they develop outwards away from the kinetochores. It seems likely that this is a progressive wave of molecular organization. This view is quite similar to that of Schrader (1932), although based upon different evidence. However, it is fundamentally distinct from that of Belar (1929) who supposed a very different relationship between traction and continuous fiber. Further tentative conclusions on the growth of traction fibers have already been given in describing the experimental results.

The extension of the attachment region in chromosomes subjected to relatively low pressures indicates a real pull by or through the traction fibers. It is almost impossible to imagine that it could be due to "repulsive forces" between the kinetochores for, if that was so, the extension should continue to increase with progressively higher pressures which further soften the chromosomes. Instead, we find the extension to be subnormal while we still have evidence of traction fibers and anaphase movement (at 4,000 lbs. in the first meiotic division). Our conclusion, then, is that the traction fiber is a reasonably stiff gel. No doubt it progressively loses rigidity with increasing pressure, but it has a margin of strength, and there is no important weakness until a pressure threshold is passed. The extension of the attachment stalk is therefore thought due to a pressure effect upon the chromosome itself so that it is softened, and can be unduly pulled out. The subnormal extension at 4,000 lbs. indicates a significant weakness of either the traction fiber or available force. It is interesting for comparison that the centrifuging experiments of Shimamura (1940) with comparable material (*Lilium* PMC) also lead to the conclusion that the traction fiber is a fairly stiff gelled structure. The latter's work seems to the author to be quite conclusive.

Chromosome structure

It seems obvious that some portion of the condensed chromosome tends to be softened, and finally liquefied, by hydrostatic pressures. Since there was no apparent effect upon uncondensed chromosomes, or upon the spirally coiled chromonemata, the portion affected would seem to be the "matrix" (no morphologically separable "sheath" is visible, and presumably more than a sheath would be involved when the attachment region is extended).[2]

A critical analysis of the data, however, discloses some relationships that cannot yet be interpreted with any assurance of certainty. The normal presence of an attachment stalk, and its further extension under relatively low pressures, suggests that the rigidity of the matrix is normally low, but is further reduced by pressure. One might suppose it to be viscous rather than a stiff gel. While the spindle gels are liquefied by moderate pressures, the matrix is not entirely liquefied until pressures of about 15,000 lbs./in.^{2} are applied when the chromosomes so fuse that they lose their visible identity. Thus a structural viscosity appears to persist and withstand very considerable pressures.

It is a fair assumption that the spindle gels obey Marsland's (1939) law, so that their rigidity is reduced 24 per cent by each pressure increment of 1,000 lbs./in.^{2} Liquefaction then occurs at a critical pressure, when gel linkages tend to break more rapidly than they can be formed. Whereas we can probably apply Marsland's law to the spindle gels, it does not seem applicable to the chromosome matrix, unless we assume that the matrix material has a much lower pressure/rigidity constant than cytoplasmic or spindle gels, i.e., much less than 24 per cent per 1,000 lbs./in.^{2} That other different gels *in vitro* do, in fact, have different constants has been demonstrated by Marsland and Brown (1942).

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[2] In the first paper of this series (Pease, 1941) chromosome aggregation was described in *Urechis* eggs subjected to hydrostatic pressure. The cytological appearance suggested that a "sheath" was involved in this fusion rather than the matrix. The *Urechis* chromosomes were so small, though, that the details were not visible. In view of the present work it seems more likely that the matrix as a whole was involved.

There is yet another aspect of chromosome structure to be considered. Why is it that with increasing pressures we find chromosomes rounding up and tending to fuse into a single mass? This looks like an interfacial phenomenon to be explained on the basis of surface tension laws. We do not observe this with uncondensed chromosomes. The author does not see how these and related observations can be explained except by the assumption that a true interface does exist between condensed chromosome and surrounding protoplasm (cf., Hirschler, 1942). Many workers do not believe that there is an osmotically active membrane separating chromosome from protoplasm, although this could explain many of the observations of chromosome swelling. However, a real interfacial boundary would not necessarily imply an osmotically active system.

In any case, it can be presumed safely that any intracellular interface would exert only a very low tension, certainly not more than a fraction of a dyne, or the very few dynes, that have invariably been recorded for water/cell interfaces, or intracellular oil/protoplasm interfaces (cf., Harvey and Shapiro, 1934 and Harvey and Schoepfle, 1939). The presence and properties of dissolved proteins would always prevent high values. Thus any interfacial tension at the surface of a chromosome would be so low that complete rounding of the aspherical shape would occur only when both chromosome and surrounding protoplasm were essentially fluid, and practically without structural viscosity. It is only at a pressure of about 15,000 lbs./in.^{2} that the observed effect indicates these conditions as being nearly fulfilled.

The spindle in chromosome movement

It has already been pointed out that there is a direct and definite correlation between anaphase movement and the presence of a good visible spindle. Hence, our outstanding conclusion is that the presence of gel structure in a spindle is essential for anaphase movement. When the gel rigidity is sufficiently reduced, the movement necessarily ceases. Other types of experimentation have less directly led to the same conclusion (cf., particularly the work of von Möllendorff, 1938 and 1939, on the specific effects of chemical agents). On the other hand, hypotheses involving attractive or repulsive forces are well nigh incompatible with the results. It is hard to imagine hydrostatic pressure affecting such forces, particularly in the low pressure range. Under pressure, with conditions of reduced viscosity, the chromosomes should move apart all the more rapidly and easily if such forces were involved. Furthermore, since Marsland's law relating pressure and viscosity expresses a logarithmic relationship, the effect should be most noticeable in the low pressure range. Obviously this is in direct disagreement with the present findings.

But what is the role of gel structure in anaphase movement? Certainly there are at least two separable structures to be considered—the traction fibers and the spindle mass.

Considering the traction fibers first, Cornman (1944) in a thought-provoking review comes to the conclusion that they are contractile structures and supply the force for movement. However, Cornman ignores one major difficulty in his otherwise excellent analysis. No one has yet been able to demonstrate that traction fibers thicken as they shorten, although this would be expected if we were dealing with contractile bodies. The author has certainly seen no evidence of this in his own preparations, nor has he been able to observe the converse of any visible thinning when a traction fiber was extended all the way across the cell from one pole to the other. We, therefore, seem to require a different explanation.

It is the author's thought that Schrader (1932) was correct in regarding traction fibers as being no more than passively semi-elastic structures. This has been given excellent experimental foundation by Ris (1943) who has been able to measure directly anaphase movement in living cells (insect spermatogonia and spermatocytes). In some cases he has demonstrated that anaphase movement is very definitely a two step process. The first, relatively rapid movement can be explained as due to the release of elastic tension so that the traction fibers do actually shorten. The remaining movement is then due to the spindle mass, with the traction fibers serving simply as passive connections to the chromosomes. Lewis (1939) produced an accelerated motion picture of dividing fibroblasts *in vitro* which beautifully showed the same phenomenon, although he has not commented upon it.

A general hypothesis of anaphase movement can be advanced on the assumption that the traction fiber is anchored at one end to the chromosome, and along some of its length to the larger gelled mass of the spindle which, in turn, is in motion. Thus it is simply a more or less elastic connection from the spindle body to the chromosome—a rope, so to say, between the machine and the load. This interpretation forces our attention to the body of the spindle.

The analysis of anaphase movement by Belar (1929) does much to delimit the problem, even though we cannot accept his general hypothesis. He demonstrated that it was impossible to account for the total movement on the basis of simple swelling or elongation of the main spindle mass (or, more specifically, the Stemmkörper). There is, however, an obvious way to avoid the difficulties outlined by Belar (other than his own solution), and still be consistent with his findings and other knowledge.

It is proposed that motion and force may be imparted to the spindle mass by means of two phase transformations. The postulate supposes that gel material is added either in the interzonal region[3] or along the greater part of the spindle, while a proportional solation occurs at the poles. Thus a material circulation is established, but a circulation by means of sol-gel-sol transformations rather than within a single phase. Actually a somewhat comparable idea has been proposed by Wassermann (1929 and 1939). Such an idea would be regarded by many as entirely too speculative, and not subject to either proof or disproof. The author, however, wishes to point out some comparable effects which are not likely to be known to most cytologists.

Dan *et al.* (1938 and 1940) discovered a remarkable phenomenon in dividing sea urchin eggs. After the furrow completes its intrusion, an entirely new region of gelled cortex is added in the center of the furrow region as the original cortical material backs out. Pease (1943) calculated that this *de novo* cortex came to cover about 11 per cent of the cell surface. This gel growth is obviously analogous to a system that could very well work within a spindle.

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[3] Note that Schmidt (1939) did not find birefringence with polarized light in the mid-region of sea urchin egg spindles, and that Shimamura (1940) found this to be the "weak" region in centrifuging experiments upon *Lilium* PMC.

Since the advent of hydrostatic pressure techniques, it has also become clear that all sorts of other cell processes involving movement are dependent upon gel structure. Thus amoeboid movement, cyclosis, streaming, cytoplasmic division, the movement of pigment granules, and the pole cell nuclei of *Drosophila* eggs, and even sperm penetration both through the egg surface and also to their final central position all cease (reversibly) when the gel is liquefied. All of these movements depend upon the rather unexpected, and admittedly little understood, properties of protoplasmic gels. Obviously the gel rearranges itself, and is itself in motion (cf., the review of Marsland, 1942). No doubt gel-sol transformations are usually if not always involved along with the rearrangement. Thus we do find empirically a common denominator for all movements other than such specialized activities as muscle contraction and ciliary motion. The author believes that a general theory of anaphase movement is in sight, and that it will come from a better physicochemical understanding of protoplasmic gel-sol systems.

SUMMARY

Hydrostatic pressures have been applied to *Tradescantia* pollen mother cells as a technique for studying the structure of division spindles and chromosomes and the mechanics of anaphase movement. The procedure has given pertinent information by virtue of the fact that increasing pressures progressively reduce gel rigidity. Sufficiently high pressure results in liquefaction. Yet the effects are reversible.

The spindle of the first meiotic division was but slightly affected by 4,000 lbs./in.^{2} pressure, yet was mostly liquefied by 5,000 lbs. The spindle of the second meiotic division withstood about 2,000 lbs. more pressure. The somatic cells were even more resistant.

Condensed chromosomes were significantly softened by even 1,000 lbs./in.^{2} pressure as indicated by an undue elongation of the kinetochore stalk. Fusion bridges became particularly obvious when 3,000 lbs. was applied. Significant shortening and rounding occurred at 4,000 lbs. Total fusion and rounding, indicating complete liquefaction of the matrix, did not occur until pressures of 15,000 lbs./in.^{2} were applied. The fusion and rounding appeared to be a surface tension effect, and suggested the existence of a true interfacial membrane between condensed chromosome and cytoplasm. Not even these highest pressures, however, affected the uncondensed prophase chromosomes so that the effect of pressure was thought to be only upon the matrix material.

Chromosome movement was limited to those pressures which did not liquefy the spindle. The presence of fusion bridges, however, resulted in very abnormal movement.

After the release of high pressures, spindles re-formed. That these were *de novo* structures was indicated by their sometimes abnormal orientation, by the frequency of multipolar spindles, and by abnormalities in the course of traction fibers. Thus, the traction fibers of two homologous chromosomes might go to a single pole. Abnormalities made it seem likely that the growth of traction fibers was in a large measure independent of the growth of the body of the spindle. The direc-

tion of growth of the traction fiber was not specifically oriented until it reached the oriented bulk of the spindle.

Chromosome movement in recovery material was abnormal in that the fusion bridges persisted. Thus the chromosome matrix which had been liquefied, had become highly viscous once more. Under such circumstances, homologous chromosomes frequently went to a single pole, and the traction fiber to the other pole extended all the way across the cell. However, such traction fibers were not thinner than normal.

The outstanding conclusion is that a gel structure in the spindle is essential for anaphase movement. The traction fiber apparently serves as nothing more than a semi-elastic connection between the chromosome and the main mass of the spindle which, in turn, is in motion. It is suggested that motion and force is imparted by means of sol-gel-sol transformations, with gel being added to the central bulk of the spindle while a proportional solation goes on at the poles.

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THE COMPARATIVE DISTRIBUTION OF TWO CHROMATOPHOROTROPIC HORMONES (CDH AND CBLH) IN CRUSTACEAN NERVOUS SYSTEMS

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INTRODUCTION

It was demonstrated by Brown (1933) that sea-water extracts of the crustacean central nervous organs contained material having a definite and characteristic effect upon certain chromatophores of the body. The nervous organs were the only tissues of the body other than the eyestalks, with their included sinus glands, that yielded such a chromatophorotropically active substance, thus suggesting that the former possibly contained a source or sources of normal, color-changing hormonal material. In the shrimp, *Palaemonetes,* injection of extracts of the nervous system were shown to bring about a rapid blanching of dark-colored specimens through concentration of the red and yellow pigments within the chromatophores, an action similar to that which could be induced by extracts of the sinus gland of the eyestalk.

Similar activity of the nervous system was described by Hosoi (1934) for *Penaeus japonicus* and by Hanström (1937) for *Penaeus brasiliensis.* Knowles (1939) found that extracts of the central nervous system of *Leander adspersus* caused concentration of the white pigment within that species. Concentration of white pigment by extracts of central nervous system was also reported for *Cambarus* by Brown and Meglitsch (1940) who worked with the chromatophores in isolated pieces of integument. Sinus gland extracts had an antagonistic action upon this pigment, thus proving that the sinus glands and nervous system did not yield exclusively identical chromatophorotropic substances.

Evidence that the central nervous organs contained sources of hormones normally involved in the adaptive color-changes of *Palaemonetes* was presented by Brown (1935) who found that any vigorous stimulation of the cut ends of the optic nerves in darkened eyestalkless specimens would induce a blanching characteristic of that following injection of extracts of central nervous organs. Koller (1930) had also observed comparable responses of eyestalkless *Crago* but did not at that time consider the central nervous organs to be a source of the active material.

More convincing evidence for the production of a normal chromatophorotropic hormone in the crustacean nervous system was presented by Brown and Ederstrom (1940). Their observations concerned the reactions of the particularly sensitive melanophores in the telson and uropods of the shrimp, *Crago.* Amputation of the eyestalks of a white-adapted animal brought about, within 3–6 minutes, a complete dispersion of black pigment in the melanophores giving the animal a "black-tailed" appearance. The condition persisted for about an hour whereupon the pigment returned to its former concentrated state, the latter condition typically lasting for several days. Brown and Ederstrom found that the black pigment could be caused to disperse again by stimulation of the eyestubs or by the injection of extracts of the circumoesophageal connectives. Upon more extensive experimentation they concluded that the mid-region of the connectives, including the connective ganglia, contained the origin of the *Crago* tail-darkening hormone (CDH) involved here. The results of these investigators were confirmed and extended when Brown and Wulff (1941) gave evidence for a second chromatophorotropic principle within the central nervous system, namely a *Crago* body-lightening hormone (CBLH) by describing that strong stimulation of the eyestubs simultaneously darkened the telson and uropods and lightened the remainder of the body, an action duplicated by injection of extracts of the central nervous system as a whole. It was shown that these two actions were due to two separable principles in that injection of ethyl-alcohol extracts of the nervous system gave only body-lightening action, the tail-darkening principle remaining in the alcohol-insoluble residue, and, that mild stimulation of the eyestubs of eyestalkless animals produced both tail-darkening and body-darkening. Brown and Wulff speculated that CDH was, in the absence of CBLH, a general body-darkening principle. This hypothesis was more specifically set forth and given experimental support by Brown (1946) who clearly demonstrated the source of this darkening principle to lie, not in the circumoesophageal connectives proper, but in the minute tritocerebral commissure interconnecting the connectives immediately posterior to the oesophagus. Injection of sea-water extract of this commissure in various experiments produced in every case tail-darkening but various degrees of either body-lightening or body-darkening. The variable effects upon the body seemed reasonably explained in terms of varying concentrations of an antagonistic body-lightening principle.

In the following experiments a survey was made of the effects of sea-water extracts of the central nervous systems of thirteen species of higher crustaceans representing the *Isopoda*, *Natantia*, *Astacura*, *Anomura*, and *Brachyura* upon *Crago* color-change. The distribution of both the *Crago* tail-darkening hormone, CDH, and the *Crago* body-lightening hormone, CBLH, was considered. We have concerned ourselves primarily with the presence or absence of each substance within the central nervous systems and, when the hormones are present in a particular species, with a survey of the relative concentrations of the principles within the parts containing the hormone in question.

Experiments and Results

The experiments to determine the distribution of CDH and CBLH were conducted in the following manner. Animals for use in assaying the concentration of active principles in extracts of nervous tissue were first prepared. The eyestalks of a number of *Crago septemspinosus*, ranging from 3–6 cm. in length, were amputated by means of a sharp scalpel and the eyestubs cauterized with an electric cautery needle. No animals were used for assay purposes until at least twelve hours following this operation, at which time they could best be described as possessing mottled black and white bodies and light telson and uropods (see Fig. 1*A*, control).

A relatively simple but effective method was used in the preparation of central-nervous-system extracts. The donor of the nervous tissue first had eyestalks removed and stubs cauterized in the same manner as described above for *Crago*. The dorsal portion of the exoskeleton was then cut away. After removing surrounding viscera and muscles the nervous organs were removed under a dissecting microscope by carefully severing the nerves about the brain, thoracic and abdominal cords and gently lifting the entire system out of the animal. Particular caution was observed in the removal of the circumoesophageal connectives so as to prevent any damage to the tritocerebral commissure. The nervous system was then placed in a watchglass containing a small amount of sea-water and divided by means of a sharp scalpel into the desired portions which usually comprised brain, connectives, thoracic cord, and abdominal cord.

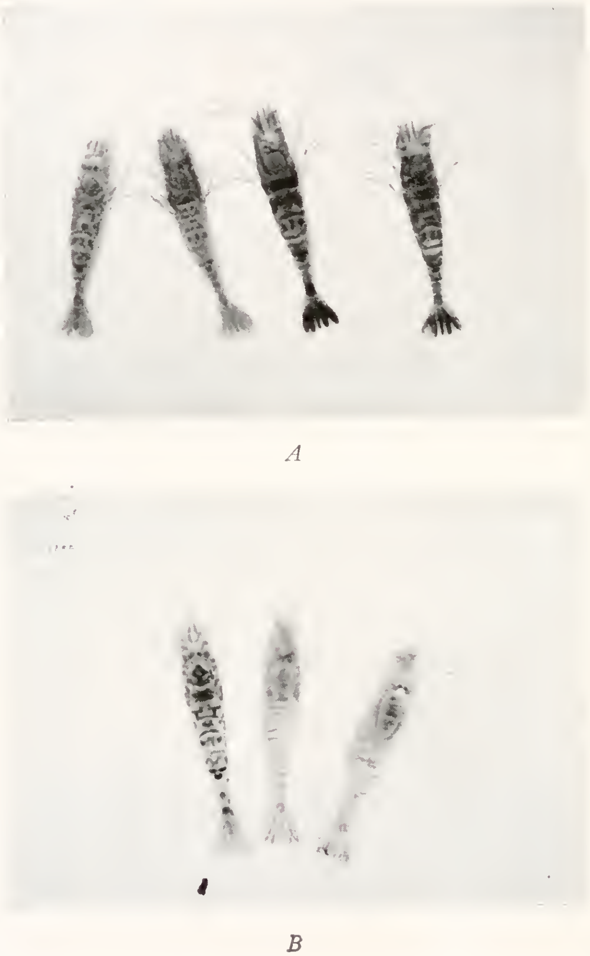

FIGURE 1. *A*. Darkening of eyestalkless *Crago* following injection of a sea-water extract of the abdominal nerve cord of *Homarus* (conc. = 1 cord/0.5 ml. sea-water). The two specimens on the left are two uninjected ones used for a control. The injections for the animals on the right were made 15 min. before the photographs were made. *B*. Lightening of eyestalkless *Crago* following injection of a sea-water extract of the circumoesophageal connectives of *Uca* (conc. = 3 pr. conn. to 0.2 ml. sea-water). The two specimens on the right were injected 8 minutes before the photographs were made.

Following this procedure the organs were transferred to individual glass mortars where excess sea-water was removed and the tissues allowed to dry partially. The tissue was then triturated with a measured amount of sea-water varying in quantity with the different species from 0.1–0.5 cc. per portion depending upon the size of the nervous system as a whole. In some cases, such as that of *Idothea,* it was necessary to use the parts of several nervous systems in the preparation of each extract in order to obtain adequate concentration and amount for assay. All extracts were centrifuged for three minutes at approximately 3,500 R.P.M. and the supernatant liquid of each injected into the dorsal musculature of the abdomen of at least two test-animals prepared as described above. The amount of extract injected into each varied with the size of the test-animal, but was normally between 0.025 and 0.04 cc. Sea-water injected or uninjected controls were observed simultaneously with all test-animals.

Table I

Responses of eyestalkless Crago to injection of extracts of various portions of the central nervous system of other crustaceans. No. of cases signifies the number of donors

			Tail-darkening Time (min.)							Body-lightening ⊖ or darkening ⊕ Time (min.)						
Species	Organ	No. cases	0	5	10	15	30	45	60	0	5	10	15	30	45	60
Homarus	Brain	7	0.0	3.3	3.6	3.9	3.7	3.7	1.6	0.0	0.0	+0.7	+2.0	+2.6	+1.9	+0.5
	Connectives	8	0.0	1.9	2.3	2.3	2.3	2.1	1.4	0.0	−1.4	−1.0	0.0	+0.6	+0.4	0.0
	Thoracic cord	8	0.0	1.6	2.1	2.2	2.8	2.7	2.5	0.0	−0.8	−0.3	+0.4	+2.8	+2.8	
	Abdominal cord	2	0.0	1.0	1.5	2.5	3.0	2.5	1.5	0.0	+3.0	+3.5	+4.0	+4.0	+4.0	+4.0
Cambarus	Brain	10	0.0	3.4	3.4	3.4	2.6	1.3	0.4	0.0	+0.8	+1.2	+1.7	+0.9	+0.4	+0.2
	Connectives	10	0.0	2.5	2.9	3.2	2.2	0.9	0.3	0.0	−1.7	−0.9	−0.6	−0.1	+0.1	+0.3
	Thoracic cord	10	0.0	2.7	3.1	3.4	2.9	2.1	1.3	0.0	−1.9	−1.7	−0.9	+0.1	+0.3	0.0
	Abdominal cord	10	0.0	2.6	2.8	2.8	2.5	1.4	1.0	0.0	+0.8	+1.2	+1.4	+1.1	+0.6	+0.2
Upogebia	Brain	7	0.0	1.3	2.3	2.3	2.2	0.8	0.0	0.0	−2.0	−1.7	−1.5	−0.4	−0.2	0.0
	Connectives	6	0.0	0.2	0.6	0.0	0.0	0.0	0.0	0.0	−2.3	−2.6	−2.0	−1.7	−0.4	−0.2
	Thoracic cord	7	0.0	1.5	2.1	2.7	2.5	2.0	1.4	0.0	−0.2	+0.7	+1.2	+0.8	+0.5	+0.2
	Abdominal cord	7	0.0	1.0	1.4	1.4	1.2	0.9	0.4	0.0	−0.6	−0.6	−0.3	0.0	0.0	0.0
Pagurus	Brain	8	0.0	0.4	0.5	0.4	0.2	0.2	0.0	0.0	−1.6	−1.6	−1.1	−0.5	−0.1	0.0
	Connectives	8	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	−1.8	−1.6	−1.0	0.0	+0.1	+0.1
	Thoracic cord	8	0.0	1.9	2.9	3.1	2.4	1.4	0.2	0.0	−1.3	−1.3	−1.3	−0.5	+0.2	0.0
Emerita	Brain	8	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	−1.5	−0.6	−0.5	−0.2	0.0	0.0
	Connectives	8	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	−1.4	−0.6	−0.2	0.0	0.0	0.0
	Thoracic cord	8	0.0	1.0	1.6	1.6	1.0	0.5	0.1	0.0	−0.9	−0.6	−0.1	−0.1	0.0	0.0
Libinia	Brain	7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	−2.6	−2.8	−2.0	−1.0	−0.3	0.0
	Connectives	7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	−1.7	−1.8	−1.7	−0.4	−0.1	0.0
	Thoracic cord	7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	−1.7	−1.7	−1.8	−0.9	−0.4	0.0

Observations of the color changes in both body and tail were taken at five-minute intervals up to fifteen minutes and at fifteen-minute intervals thereafter. The degree of darkness of the tail or body was described within the range, + 1 to + 4, the number + 4 representing the maximum extent of darkening and the number + 1, the minimum observable one. In a similar manner body-lightening was indicated by the range, − 1 to − 4, with − 4 denoting the greatest extent of body-lightening. Final results for a number of experiments were averaged and are presented in tabular form in Table I. These results have been further analyzed so as to present the distribution of CDH and CBLH within the central nervous system of each of the species considered (see Tables II and III). In these tables, the relative distribution of activity of the hormones is calculated for the various portions of the nervous system for each species.

This was done as follows. The average values of the chromatophores at 5, 10, 15, and 30 minutes following extract-injection were of themselves averaged. Then for Table II the portion of the nervous system producing maximum darkening was arbitrarily given the value 1.00, the activity of the other parts being expressed in terms of simple proportions of this. For Table III the part showing maximum lightening was given the value − 1.00 with the activity of other parts similarly expressed proportionately. The positive values in the latter table obviously indicate darkening rather than lightening.

TABLE II

The quantitative distribution of CDH activity within the central nervous systems of a number of crustaceans. The region of maximum activity is arbitrarily given the value 1.00. It is important to note that each portion of the nervous system, regardless of size, is extracted in an equal volume of sea-water, and the relative concentrations of the principles investigated are expressed solely in terms of their activities. This note applies equally to Table III.

Classification	Species or genus	Brain	Connectives	Thoracic cord	Abdominal cord
Isopoda	*Idothea baltica*	1.00	1.00	1.00	1.00
Decapoda *Natantia*	*Crago septemspinosus*	some 0.06	1.00	0.22	0.21
	Palaemonetes vulgaris	0.85	1.00	0.97	0.98
Reptantia *Astacura*	*Homarus americanus*	1.00	0.61	0.61	0.53
	Cambarus virilis	1.00	0.84	0.94	0.84
Anomura	*Upogebia affinis*	1.00	0.10	0.80	0.65
	Pagurus sp.	0.17	0	1.00	—
	Emerita talpoidea	0	0	1.00	—
Brachyura *Oxyrhyncha*	*Libinia* sp.	0	0	0	—
Brachyrhyncha	*Cancer irroratus*	0	0	0	—
	Carcinides maenas	0	0	0	—
	Ovalipes ocellatus	0	0	0	—
	Uca pugilator	0	0	0	—

Within the single species of *Isopoda* investigated, *Idothea baltica*, there appears to be roughly a uniform distribution of CDH throughout the central nervous system, all organs darkening the telson and uropods of *Crago* to approximately the same degree. Great variations in distribution of the hormones occur among the decapods. The Natantian, *Crago* apparently possesses significant CDH activity only in the regions of the circumoesophageal connectives. CDH is differentially distributed throughout the central nervous system of the anomurans with highest quantity usually in the posterior region of the thoracic cord, is relatively uniformly distributed within the central nervous system of the astacurans and *Palaemonetes,* and is entirely absent within that of the brachyurans.

The quantitative distribution of CBLH was considered here solely within the reptantian nervous system, although it is known to be present throughout the central nervous system of the natantians (Brown and Wulff, 1941). Both the anomurans and brachyurans show wide distribution of this principle throughout brain, connectives, and thoracic cord. However, a striking feature is noted in the astacurans and the natantian, *Palaemonetes,* in which a darkening (see Fig. 1*A*), as well as a lightening, of the body occurs.

Table III

The quantitative distribution of CBLH activity within the central nervous systems of a number of crustaceans. The region of maximum body-lightening is arbitrarily assigned the value − 1.00. The + values indicate body-darkening.

Classification	Species or genus	Brain	Connectives	Thoracic cord	Abdominal cord
Isopoda	*Idothea baltica*	pres.	pres.	pres.	pres.
Decapoda					
Natantia	*Crago septemspinosus*	pres.	pres.	pres.	pres.
	Palaemonetes vulgaris	pres.	pres.	pres.	pres.
Reptantia					
Astacura	*Homarus americanus*	+2.40	−1.00	+2.20	+7.20
	Cambarus virilis	+1.09	−0.73	−1.00	+1.00
Anomura	*Upogebia affinis*	−0.64	−1.00	+0.27	−0.18
	Pagurus sp.	−0.92	−0.85	−1.00	
	Emerita talpoidea	−1.00	−0.86	−0.57	
Brachyura					
Oxyrhyncha	*Libinia* sp.	−1.00	−0.67	−0.71	
Brachyrhyncha	*Cancer irroratus*	pres.	pres.	pres.	
	Carcinides maenas	pres.	pres.	pres.	
	Ovalipes ocellatus	pres.	pres.	pres.	
	Uca pugilator	pres.	pres.	pres.	

The two species of astacurans with which we have concerned ourselves more or less parallel one another with respect to the distribution of CDH. In *Homarus* and *Cambarus* the region of greatest quantity of this principle is the brain, and is followed by an apparent gradual diminution of the substance from anterior to posterior within the nervous system. The problem of CBLH distribution seems somewhat more complex since, as has been previously mentioned, certain of these nervous-system extracts appear to produce body-darkening preceded by a body-lightening. The abdominal-cord extract is particularly active in body-darkening and only the connectives and thoracic cords of *Homarus* and *Cambarus* show any body-lightening activity at all. In these cases where body-lightening is indicated, the lightening persists for only a short time and is followed by a definite darkening. These observations suggest that the body-darkening activity observable for extracts of the astacuran central nervous system is explainable in terms of CDH. It is significant that in no case is body-darkening ever obtained from a portion of the nervous system lacking tail-darkening activity. However, since there is no essential direct correlation between the degree of tail-darkening and the degree of body-darkening even within a single species, the observed results must be the consequences of varying proportions of the two principles within the extracts, with the degree of influence of either one being a function of its relative concentration at any given instant.

There are significant differences in the distribution of CDH within the group of anomurans. *Pagurus* and *Emerita* exhibit similar tail-darkening activities and these are shown chiefly by thoracic cord extracts. On the other hand, extracts of brain, thoracic and abdominal cords of *Upogebia* all contain notable amounts of CDH. Another similarity between extracts from *Pagurus* and *Emerita* is seen in the distribution of CBLH. CBLH is found in considerable amounts in brain, connectives, and thoracic cord of both genera. However, the thoracic cord extracts of *Upogebia* show almost a complete absence of CBLH activity while the extracts of the remaining parts of the central nervous system produce a definite body-lightening, the connectives being most active in this respect.

The absence of CDH within the brachyurans investigated as well as the restriction of this principle to the connective region of the natantians studied confirms the results of Brown and Ederstrom (1940). Experimental data show that moderate amounts of CBLH are found in brain, thoracic cord, and connectives. Although results for CBLH distribution for brachyurans are shown only for *Libinia*, it has been found that they are qualitatively the same for *Uca*, *Cancer*, *Carcinides*, and

TABLE IV

The responses of eyestalkless Crago to injections of extracts of parts of the thoracic cord of some anomurans, showing the differing distributions of CBLH and CDH activity. No. of cases signifies number of donors.

			Tail-darkening Time (min.)							Body-lightening ⊖ or darkening ⊕ Time (min.)						
Species	Part of thor. cord	No. cases	0	5	10	15	30	45	60	0	5	10	15	30	45	60
Pagurus pollicaris	Anterior ¼	8	0.0	0.3	0.3	0.4	0.3	0.0	0.0	0.0	−2.3	−2.2	−2.0	−0.6	−0.3	0.0
	Second ¼	8	0.0	0.6	0.7	0.7	0.2	0.0	0.0	0.0	−0.7	−0.6	−0.3	−0.3	0.0	0.0
	Third ¼	8	0.0	1.5	1.6	1.6	0.5	0.5	0.4	0.0	−0.4	−0.4	−0.3	0.0	0.0	0.0
	Posterior ¼	8	0.0	2.6	2.6	2.4	0.9	0.5	0.0	0.0	−0.5	−0.5	−0.3	−0.1	0.0	0.0
Pagurus longicarpus	Anterior ¼	6	0.0	0.5	0.5	0.5	0.0	0.0	0.0	0.0	−1.7	−1.6	−1.2	−0.4	−0.2	0.0
	Second ¼	6	0.0	0.2	0.2	0.2	0.0	0.0	0.0	0.0	−1.0	−1.0	−0.3	0.0	0.0	0.0
	Third ¼	6	0.0	1.3	1.4	1.5	0.8	0.5	0.2	0.0	−1.2	−0.8	−0.2	0.0	+0.2	+0.2
	Posterior ¼	6	0.0	2.0	2.0	2.0	1.2	0.5	0.0	0.0	−0.5	−0.4	−0.2	−0.2	0.0	0.0
Emerita talpoidea	Anterior ⅕	4	0.0	0.8	0.8	0.8	0.3	0.0	0.0	0.0	−2.3	−2.3	−1.8	−0.5	0.0	0.0
	Second ⅕	4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	Third ⅕	4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	−0.3	−0.3	0.0	0.0	0.0	0.0
	Fourth ⅕	4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	−0.5	−0.5	0.0	0.0	0.0	0.0
	Posterior ⅕	4	0.0	0.3	1.8	1.8	0.3	0.1	0.0	0.0	−0.3	+0.8	+0.8	0.0	0.0	0.0

Ovalipes. The striking body-lightening effect of a strong extract of *Uca* connectives and commissures is illustrated in Figure 1*B*.

An attempt was made to analyze further the localization of CDH and CBLH within the thoracic cords of *Emerita* and two species of *Pagurus: pollicaris* and *longicarpus* (Table IV). The procedure consisted of dividing the thoracic cords into a number of approximately equal portions, four in the case of *Pagurus* and five in that of *Emerita.* It was observed that the concentration of CDH within the thoracic cord of both *P. pollicaris* and *P. longicarpus* is greatest in the posterior fourth of the cord and decreases gradually along the cord as one proceeds anteriorly. In *Emerita* the highest region of CDH concentration is also the posterior portion of the thoracic cord. However, there is a lack of CDH in any of the central portions of the thoracic cord in *Emerita.* It would seem then that the distribution of CDH in the thoracic cord of *Emerita* is more restricted than in *Pagurus.*

The distribution of CBLH in the thoracic cord of *P. pollicaris* and *P. longicarpus* is similar. The most intense body-lightening effect is brought about by extracts of the anterior fourth of the cord while less intense reactions are produced by extracts of the remaining portions. Experiments with extracts of *Emerita* thoracic cord indicate a higher concentration of CBLH in the anterior portion of the cord, and apparent absence of CBLH in the second portion and only slight amounts of the principle in the third, fourth and fifth divisions of the cord. In summarizing the distribution of CDH and CBLH within the thoracic cords of *Pagurus* and *Emerita* we can say that CDH is relatively more concentrated posteriorly in the thoracic cord while CBLH appears more concentrated anteriorly.

DISCUSSION OF RESULTS

The effect of the extracts of the central nervous system upon the dark pigments of the telson and uropods of *Crago* possesses a characteristic pattern in each of the major groups of the order Decapoda. In the Natantian, *Crago,* we have observed the restriction of CDH activity to the circumoesophageal connectives, whereas the *Astacura* and *Palaemonetes* exhibit a more generalized occurrence of the hormone within the organs of the central nervous system. However, as one proceeds to the *Anomura,* these contain changes from the widespread condition in the astacurans to a more specialized one as evidenced by the restriction of CDH in the thoracic cord of two of the three genera examined. Finally there is an entire lack of CDH among the brachyurans.

Experimental data concerning the distribution of CBLH in the reptantians present an interesting problem. Although both the anomurans and brachyurans possess the body-lightening hormone in varying amounts throughout the entire central nervous system, the astacurans appear to limit the hormone to connectives and thoracic cord. The simplest explanation for the body-darkening activity of the astacuran central-nervous-system extracts involves action of the tail-darkening principle. It is thought that CDH produces body-darkening after CBLH has been exhausted or in the absence of CBLH. This is indicated in Figure 2 in which selected portions of the central nervous system of *Libinia, Cambarus,* and *Homarus* are shown to produce a graded series of differential effects upon the coloration of the body of eyestalkless *Crago.* These range all the way from maximum body-lightening and no trace of darkening (*Libinia* brain) through initial lightening followed by darkening, to immediate and extensive body-darkening (*Homarus* abdominal cord). These results are believed to be explained in terms of different relative amounts of CDH and CBLH. The former is known to be absent in the case of *Libinia*, and it is assumed that the latter is absent or nearly so in the case of *Homarus* abdominal cord. In the case of the extracts of *Homarus* thoracic cord and connectives and those of *Cambarus* thoracic cord, CBLH is present in small amounts and lightens the body for a short time, thereby delaying the darkening influence of CDH on the body.

A comparison of tail-darkening and body-darkening within *Crago* injected with nervous system extracts from numerous sources suggests a rough positive correlation between the two (Fig. 3). Generally speaking, we may infer from these data that the tendency towards body-darkening is greater in those animals showing a high degree of tail-darkening. This gives further support for an active role of CDH in body-darkening.

Unlike the *Decapoda* the *Isopoda* apparently exhibit a uniform distribution of CDH within the central nervous system. However, since only a single species was considered, further experimentation is deemed necessary before any decisive statement is made concerning CDH distribution within this group.

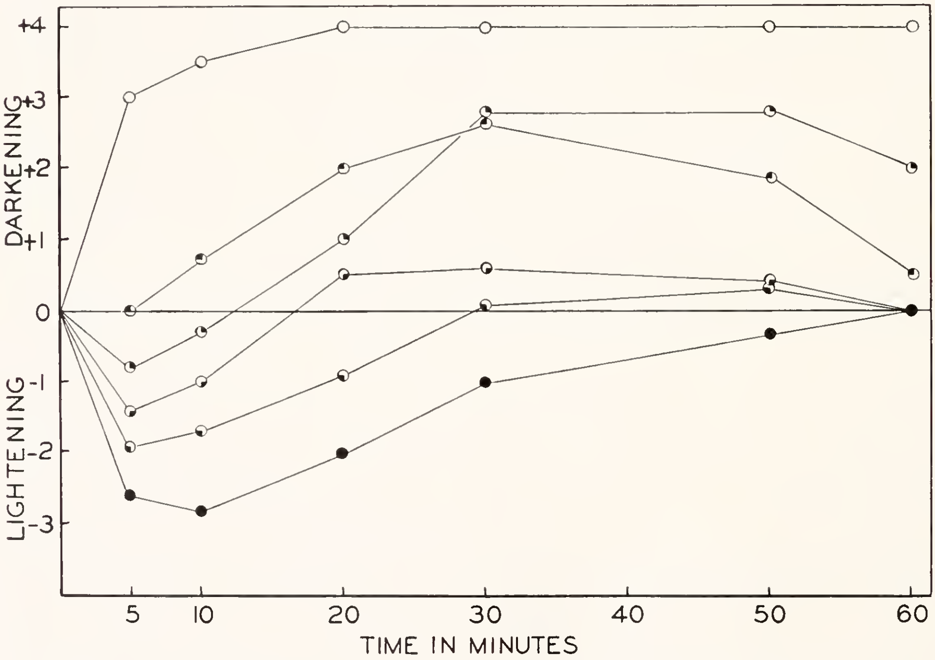

FIGURE 2. The influences of extracts of selected portions of the central nervous system of some crustaceans upon the body coloration of eyestalkless *Crago*.

From most positive to most negative at the end of 10 minutes are shown, respectively, *Homarus* abdominal cord, *Homarus* brain, *Homarus* thoracic cord, *Homarus* circumoesophageal connectives, *Cambarus* thoracic cord, and *Libinia* brain. Concentration in each experiment was: organs of one specimen/0.5 ml. sea-water.

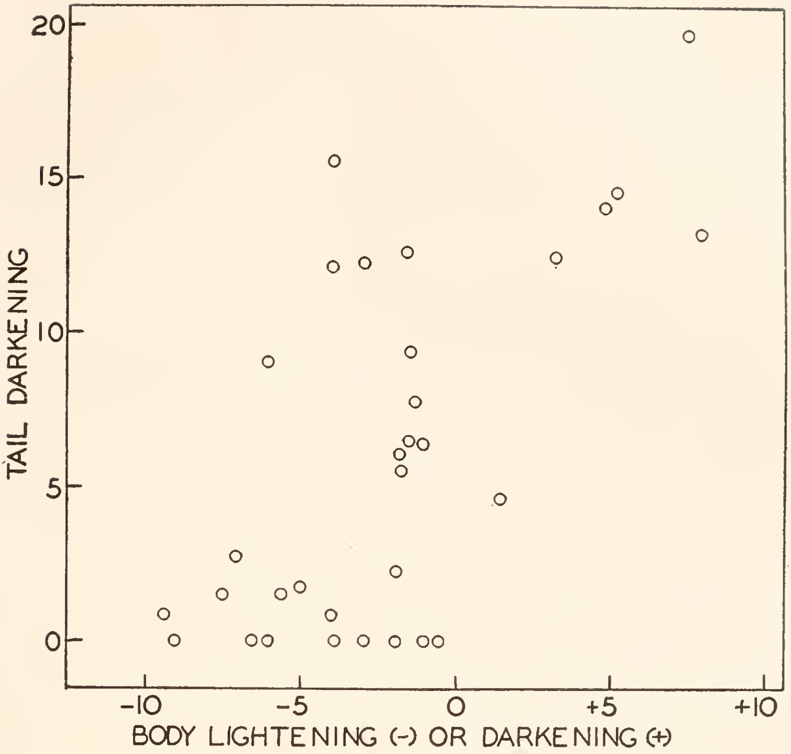

FIGURE 3. The general relationship between the degree of darkening or lightening of the body proper of eyestalkless *Crago* and the degree of darkening of the telson and uropods. Darkening of the tail is expressed as the algebraic sum of the intensities of the reactions at 5, 10, 15, 30, 45, and 60 min. following extract injection, thereby including a measure of both intensity and duration of the effect. Body-lightening, being more rapidly transitory, is expressed as the algebraic sum of the values at 5, 10, 15, and 30 min.

SUMMARY

1. A survey was made of the effects upon *Crago* color-change of sea-water extracts of various parts of the central nervous system of thirteen species of higher crustaceans. The crustaceans represented the groups *Isopoda, Natantia, Astacura, Anomura,* and *Brachyura.*

2. Extracts of various portions of the nervous system among the various groups showed wide differences in their total chromatophorotropic activities, producing various degrees of telson and uropod darkening and of body-lightening and darkening.

3. An analysis of the results gave support to the hypothesis that most crustacean nervous systems possess at least two principles, *a*) a *Crago* body-lightening principle, CBLH, lightening all portions of the body except telson and uropods, and *b*) a *Crago*-darkening hormone, CDH, darkening the telson and uropods, and, in the absence of CBLH, the body as well.

4. CBLH is more or less uniformly distributed throughout the nervous systems of all the species examined except the astacurans in which it is demonstrated only for the circumoesophageal connectives and thoracic cord.

5. CDH is restricted to the circumoesophageal connective region of the *Natantia,* is differentially distributed throughout the nervous systems of anomurans, with highest concentration in the posterior region of the thoracic cord, and is distributed throughout the nervous systems of the other species except the brachyurans in which it is absent.

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PHYSIOLOGICAL OBSERVATIONS ON WATER LOSS AND OXYGEN CONSUMPTION IN PERIPATUS[1]

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The small group of species which comprises the Onychophora have long been of interest because of their unique combination of arthropod and annelid characters which places them in a phylogenetic position intermediate to those two extensive groups (Snodgrass, 1938). They are further of interest because of their close homogeneity despite a sporadic distribution that encompasses a large portion of the world and points to an ancient separation of some of the genera (Clark, 1915; Brues, 1923). This homogeneity appears to be physiological and ecological as well as morphological,[2] since Peripatus is restricted everywhere to a moist but terrestrial environment. Further, their sporadic and fluctuating local distribution suggests that environmental variation, presumably in moisture, is actively limiting their occurrence even in the regions where they are found.

Physiological observations on members of this group, then, are of interest, and it is of particular interest to examine the process of water loss and to contrast Peripatus in this respect to comparable annelids and arthropods. Manton and Ramsay (1937) have reported a value for water loss in Peripatus (*Peripatopsis*) at 30° and with wind velocity of 7.0 m./sec. (16 m.p.h.). These conditions, however, seem rather severe for a species which is uncomfortable at temperatures above 20° (Manton, 1938) and which, living in crevices, must have little exposure to wind. The experiments reported here were made under conditions which more nearly approximate those encountered by the animal in nature.

In this connection reports in the literature suggest that there may have been some temperature adaptation in the Onychophora. Thus the two species studied here, both from Panama (lat. 9° N.), stayed in good condition at a temperature of 25° ±. In contrast as already noted, Peripatus from near Cape Town (lat. 34° S.) became uneasy at temperatures above 20° although low temperatures, even down to freezing, did not bother them. They survived very well in England (Manton, 1938; Sedgwick, 1885) as have specimens from New Zealand (lat. 40° S.). The latter were only successfully transported through the intervening tropical regions with the aid of refrigeration (Sedgwick, 1887). Peripatus from New Zealand (Hutton, 1876) and from Australia (Steel, 1896) are reported to become torpid during the winter but with no subsequent ill effects. On the other hand Sclater (1887) reported that his specimens from British Guiana (lat. 7° N.) successfully survived the trip to England, "but unfortunately were much affected by the cold, and were therefore killed."

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[1] These observations are by no means complete, but because the literature contains little data on living Onychophora, particularly on New World species, and because there was no immediate prospect of obtaining a further supply of these unusual animals, it seemed advisable to present them at this time.

[2] It should be noted, however, that for such a small group of Onychophora show remarkable diversity in their embryological development and their mode of reproduction.

MATERIAL

These Peripatus were secured on Barro Colorado Island, Canal Zone, through the great kindness of Mr. James Zetek. Two species (note Clark and Zetek, 1946) were obtained, the larger of which (*Epiperipatus brasiliensis varians*) had a contracted length of 50 mm. and was uniformly colored a rich red-brown. The smaller species (*Oroperipatus corradi*) had a contracted length of 25 mm. and was a chocolate color with lighter underside and with darker legs and a dark, median, dorsal stripe 0.3 mm. in width. The animals were taken in early September and these observations were made in Cambridge about a month later. During the interim they were kept in moist forest debris but were not given suitable food other than the supply of termites initially in the debris. The animals survived the trip well and apparently stayed in good health until just before death which presumably occurred through starvation.

The general behavior of these individuals corresponded to that described for other species (Manton, 1938; Holliday, 1942; Andrews, 1933; Steel, 1896; Sedgwick, 1885; etc.). They were retiring and preferred to remain inactive in some dark crevice. They are sensitive to light but react even more sharply to dryness which stimulates them to constant activity. The smaller species were definitely more sensitive in this respect and could not be held still even for a moment.

An occurrence involving an individual of the larger species may be of particular interest. On the occasion of mechanical injury to one of its antennae that member was placed in the mouth and the injured portion, about half the length, was removed. The stump healed and the individual did not appear to be inconvenienced by the loss. Parturition as observed in these specimens has been described elsewhere (see Morrison, 1946).

The rate of oxygen consumption and water loss in Peripatus was compared to several arthropods and annelids of fairly similar size, habitat and body form: centipeds (*Lithobius*); millipeds (*Julus*); sow bugs (*Oniscus*); and earthworms. These were all collected locally with the exception of one small tropical earthworm found among the debris.

OBSERVATIONS

Sensory responses

With the exception of the antennae the animals showed equal tactile sensitivity all over the body, on the dorsal and ventral surfaces and on the legs. A very light stimulation could be applied with no response, a light one produced a local withdrawal of a leg or small section of the body, while a strong stimulus led to a general withdrawal. Holliday (1942) noted that fairly large wood lice and centipeds could crawl over the body of a Peripatus without evoking any response. The antennae are much more sensitive and the lightest touch here results in the retraction of one or both. With stronger or repeated stimulation the animal will completely contract and change its direction of progression; further irritation provoked the well known ejection from the slime glands. These responses are in accord with the histological findings of Manton (1937) that while a single well ensconced sense capsule was found in each primary body papilla, each antennal papilla bore at least three much more exposed capsules with much heavier innervation.

The animals usually walked forwards but when startled would often reverse their direction, apparently walking backwards with equal ease. Occasionally they would half turn backwards and then move in the form of a "U" with the legs of the anterior half walking forward and those of the posterior half walking backwards. This mode of progression must impose an interesting problem in coordination.

The response of the animals to a single point source of light (a two-cell flashlight with reflector and glass removed, at a distance of 0.5 to 1 m.) was recorded by tracing their path on a large underlying sheet of paper. A number of records were made both with the light fixed and with it moved through 90 or 180° halfway through the record. Examination of the records showed no oriented negative phototropism; indeed, the animals actually travelled towards the light more often than away from it. Thus these animals would appear to be unable to localize light but only to be aware of it. This corresponds to the observations of Manton (1938) that the movement of objects near Peripatus elicited a response only when accompanied by air movement. These experiments were not carried out in a saturated environment, however, and it is possible that with the very strong stimulus of dryness removed, some phototropic pattern might be observed.

Water balance

In measuring water loss the animals were placed in large ($D = 5$ cm.) flat, weighing bottles containing a layer of calcium chloride covered by a floor of brass gauze. Measurements were made at 24° which is within the range normally encountered by these species (Kenoyer, 1929), and for periods of 30 minutes. No circulation was supplied, the movement of the animals themselves providing for convection. The Peripatus were particularly uneasy in this very dry atmosphere and kept in constant and vigorous motion.

The values obtained for the two species of Peripatus and for several other animals are summarized in Table I. Water loss has been computed on the basis of both body weight, and the two-thirds power of the body weight.[3] The latter is perhaps a more reasonable basis for comparing animals of different size. The two values for Peripatus agree well and lie between those found for the annelids and arthropods. They indicate that Peripatus has a twofold advantage over the earthworm[4] in the conservation of water; and that it is at a twofold disadvantage as compared to the centiped, the most xerosensitive arthropod studied. Other arthropods showed values ranging down to one-twentieth that observed in Peripatus. These data are presented graphically in Figure 1.

Manton and Ramsay (1937) reported on water loss in Peripatus under the much more rigorous conditions of 30° with a 7 m./sec. (16 m.p.h.) wind and a relative humidity of 27.5 per cent. They found a value of 13.0 mg./g. min. or 2 to 3 times our value. A similarly measured value for an earthworm was about half as large on a weight basis or of equal magnitude on the basis of surface area. However, the advisability of making measurements under physiological conditions should be stressed since under abnormal circumstances quite different relations may hold. Thus, for example, Ramsay (1935) showed that in the cockroach water was lost much more rapidly at temperatures above 30° with an apparent breakdown of the hydrophobic character of the body surface.

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[3] This quantity is proportional to the surface area in animals of similar body form. In Peripatus and the arthropods where the actual body surface is increased by appendages and papillae, loss of water very probably takes place largely through the trachae (note Mellanby, 1935). Water loss will therefore be related to respiration which is also roughly proportional to the two-thirds power of the body weight in animals of different size (Krogh, 1916).

[4] This will be a minimum figure since the body weight of the earthworm includes a considerable amount of dirt in the gut. These earthworms were kept in clean wet containers for 1½ days before use, during which time they evacuated up to 15 per cent of their weight, but more undoubtedly remained.

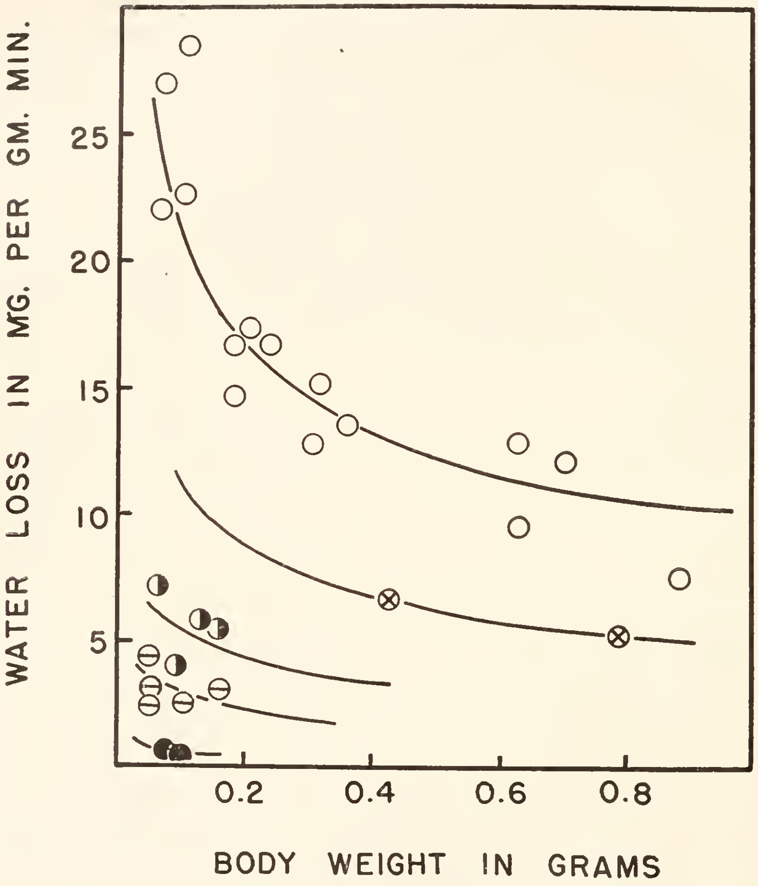

FIGURE 1. Water loss in Peripatus and other animals at 24° over calcium chloride as a function of the body weight. Open circles, earthworms; crossed circles, Peripatus; half-closed circles, centipeds; lined circles, sow bugs; closed circles, millipeds. The curves represent $Y = K\ (X)^{2/3}$, where the values for K are the average values given in Table I.

In considering this function it is of interest to note that Clark (1915) concluded on the basis of distributional and taxonomic considerations that the Onychophora had originally evolved in a cooler rather than a warmer environment. Thus, the more primitive groups are found on mountains or in the "temperate" regions while the more recent forms are tropical. This is, of course, entirely in accord with the physiological considerations since the xerotic stress would be reduced at a lower temperature and such an environment would be more favorable for evolution from an aquatic to a terrestrial mode of life.

TABLE I

Water loss in Peripatus and other animals at 24° over calcium chloride

Animal	Number and weight in mg.	Duration of experiment in min.	Water loss	
			mg./g.min.	mg./g.$^{2/3}$min.
Earthworm	884	15	7.4	7.1
	703	15	12.0	10.4
	360	15	13.4	9.6
	208	10	17.3	10.4
	105	8	22.6	10.7
Peripatus				
Epiperipatus	788	30	5.2	4.7
Oroperipatus	423	30	6.6	5.0
Centiped	150	60	5.6	3.0
	135	20	5.9	3.1
	4×95	60	4.1	1.9
	4×63	30	7.2	2.8
Sow bug	158	25	3.0	1.6
	97	40	2.5	1.2
	6×49	60	3.0	1.1
	48	20	4.5	1.6
Milliped	3×76	120	0.56	0.24
	3×98	600	0.44	0.21
Averages				
Earthworm	15 Experiments			9.9
Peripatus	2 Experiments			4.9
Centiped	4 Experiments			2.5
Sow bug	5 Experiments			1.3
Milliped	2 Experiments			0.22

Respiration

The oxygen consumption of the larger species of Peripatus and of various other animals was measured in a Warburg apparatus.[5] Carbon dioxide was absorbed in sodium hydroxide in a small cup fused to the bottom of the chamber. The animals were placed directly in the chamber and were kept from the lye by a small screen shield. Measurements were made at 25.0° C. over a period of 60 minutes.

The results on Peripatus are shown in Figure 2. After a restless initial period (10 minutes) it settled down to a very uniform rate of oxygen consumption. The centipeds, also shown in Figure 2, were less regular. The results for the various animals are summarized in Table II. The exact significance of the "resting" or "basal" oxygen consumption is not known but some correlation between it and the "intensity" of the organism has been observed. Compared on a weight basis Peripatus consumes oxygen at the same rate as the earthworm and at about half the rate of the arthropods. It has been observed, however, that within a given group, the metabolism per unit of weight varies with the size of the animal (note Edwards, 1946, for example), and that the metabolism is more nearly proportional to some lower power of the weight. As a first approximation this may be taken as the two-thirds power (Krogh, 1916). When the oxygen consumption is compared on this basis, Peripatus agrees more closely with the arthropods and has a higher value than the earthworm.

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[5] I am indebted to Dr. William Carroll for the use of his calibrated Warburg assembly.

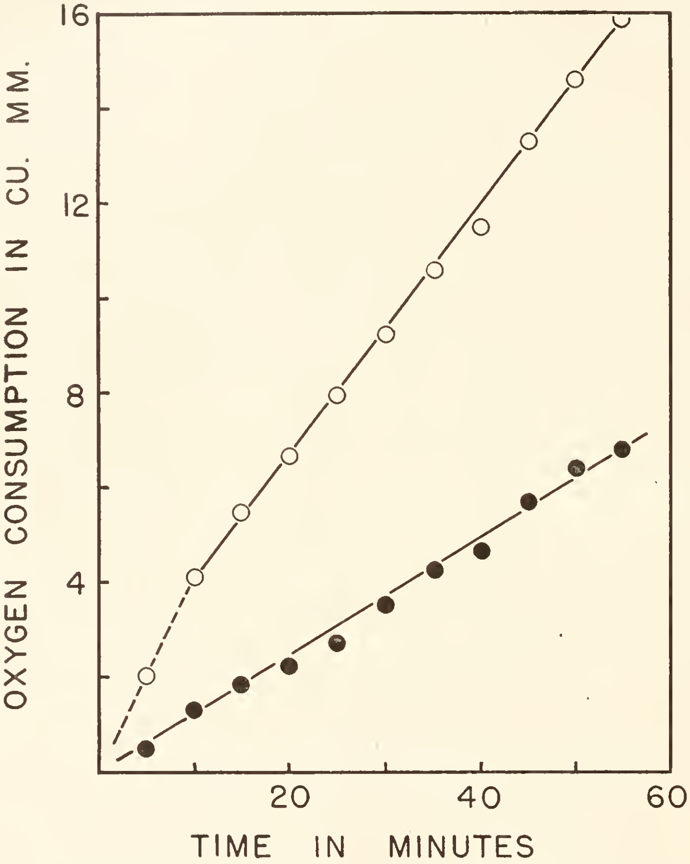

Figure 2. Oxygen consumption in Peripatus and the centiped as a function of time. Open circles, Peripatus (*Epiperipatus*), 0.68 g.; closed circles, 3 centipeds, total weight 0.33 g.; temperature, 25°.

The hydrophobic character of the body surface has been noted by many observers. It is particularly evident when the animal is submerged since the body papillae hold the water away from the body surface and leave the animal entirely surrounded by a sheath of air. It would seem entirely possible that this air sheath may function as a respiratory surface under water. Such a mechanism has been demonstrated in certain aquatic insects which carry down an air supply by means of hydrophobic hairs and which, by this means, greatly extend their periods under water (Krogh, 1941; Wigglesworth, 1931). Since Peripatus must be often covered by water in rainstorms, particularly as its lack of resistance to dessication forces it to frequent wet places, this mechanism could be of real utility and have a considerable survival value. This would provide a functional explanation for the papilla-covered body surface which is characteristic and unique in the Onychophora.

TABLE II

Oxygen consumption in Peripatus and other animals at 25° C.

Animal	Weight in mg.	Oxygen consumption	
		cc./g.hr.	cc./g.$^{2/3}$hr.
Earthworm	96	0.22	0.10[1]
Peripatus (*Epiperipatus*)	680	0.23	0.20
Millipeds	3×111	0.46	0.22
Centipeds	2×69	0.56	0.22
Pill bugs	5×61	0.35[2]	0.14

[1] Lesser (1908) reported values of 0.4 cc. per g.$^{2/3}$hr. at 19° at which temperature the oxygen consumption should be about half that measured at 25° (Vernon, 1897).

[2] Edwards (1946) reports a similar value but at a temperature of 17°.

SUMMARY

The Onychophora represent a morphological transition between the annelids and the arthropods. They also represent a physiological transition between the aquatic and the terrestrial environment. In the latter transition the most important adaptations are those involving the functions of water conservation and respiration.

The ability of Peripatus to conserve water has been compared to that of comparable annelids and arthropods. Peripatus is shown to be intermediate to those two groups in this function, losing twice as much water as the centiped, but only one-half as much as the earthworm. This corresponds to its taxonomic and ecological positions.

The "resting" rate of oxygen consumption has also been compared to other animals. The rate in Peripatus is comparable to that in the arthropods and larger than that in the earthworm.

It is suggested that the unique papilla-covered body surface may represent an adaptation for underwater respiration to meet the environmental restriction imposed by the inadequate regulation of water loss.

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STUDIES ON CILIATES OF THE FAMILY ANCISTROCOMIDAE CHATTON AND LWOFF (ORDER HOLOTRICHA, SUBORDER THIGMOTRICHA)

III. ANCISTROCOMA PELSENEERI CHATTON AND LWOFF, ANCISTROCOMA DISSIMILIS SP. NOV., AND HYPOCOMAGALMA PHOLADIDIS SP. NOV.

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INTRODUCTION

Chatton and Lwoff described in 1926 two ciliates for which they created the genus *Ancistrocoma: A. pelseneeri,* from the gills and palps of *Macoma balthica* (L.); and *A. pholadis,* from *Barnea* (*Pholas*) *candida* (L.). Their descriptions of these two species are of a preliminary nature and are not accompanied by illustrations. More detailed descriptions of *A. pelseneeri,* together with illustrations, are given in two papers of Raabe (1934, 1938).

Kofoid and Bush (1936) described as *Parachaenia myae* a ciliate from the pericardial cavity and excurrent siphon of *Mya arenaria* L. which Kirby (1941) noted was in several respects apparently identical with *A. pelseneeri.* Kudo (1946), however, listed *Parachaenia myae* as a valid species in the suborder Gymnostomata. Kofoid and Bush stated that they did not find *P. myae* in any other molluscs which were present in the same localities as the host species. I have studied the ciliate associated with *Mya arenaria* in San Francisco Bay and have compared it with similar forms from *Cryptomya californica* (Conrad), *Macoma inconspicua* Broderip and Sowerby,[1] *Macoma nasuta* (Conrad), and *Macoma irus* (Hanley) from San Francisco Bay, and from *Macoma secta* (Conrad) from Tomales Bay, California. I have concluded that the ciliate described by Kofoid and Bush as *Parachaenia myae* is not specific in *Mya arenaria* and that *P. myae* is identical with *Ancistrocoma pelseneeri* Chatton and Lwoff.

On the gills and palps of the rock-boring piddock *Pholadidea penita* (Conrad) there occurs a species of *Ancistrocoma* which is clearly distinct from *A. pelseneeri* and which I will describe in this paper as *Ancistrocoma dissimilis* sp. nov. Another ciliate I have studied from *P. penita* is referable to the genus *Hypocomagalma,* created by Jarocki and Raabe (1932) for *H. dreissenae,* from the fresh water mussel *Dreissena polymorpha* (Pall.). It will be described herein as *Hypocomagalma pholadidis* sp. nov.

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[1] By some malacologists the small species of *Macoma* referred to in this paper as *M. inconspicua* is considered to be conspecific with *M. balthica;* by others it is considered to be a subspecies of *M. balthica.* No conclusive evidence has been presented in the literature in recent years either to support or refute these contentions.

ANCISTROCOMA PELSENEERI CHATTON AND LWOFF

(Figure 1; Plate I, Figs. 1, 2)

The body is elongated and somewhat flattened dorso-ventrally.[2] As seen in lateral view, the ciliate is banana-shaped, the ventral surface being incurved. The anterior end is more or less attenuated. The body is usually widest and thickest in its posterior third. Forty living individuals taken at random from *Mya arenaria* ranged in length from 50 μ to 83 μ, in width from 14 μ to 20 μ, and in thickness from 11 μ to 16 μ, averaging about 62 μ by 16 μ by 12.5 μ. Twenty individuals from *Macoma inconspicua* ranged in length from 52 μ to 78 μ, in width from 14 μ to 19 μ, and in thickness from 11 μ to 15 μ.

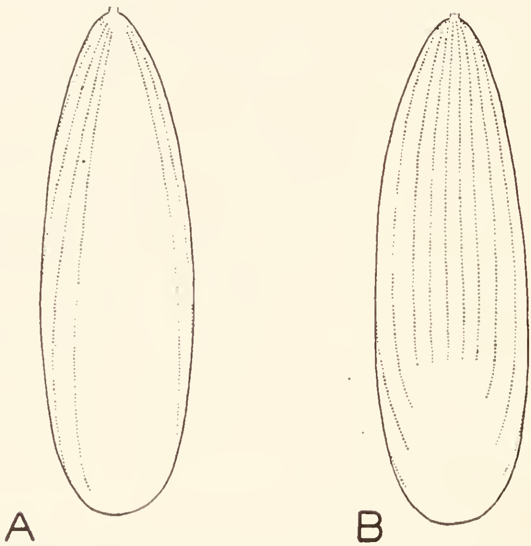

FIGURE 1. *Ancistrocoma pelseneeri* Chatton and Lwoff. Distribution of ciliary rows, somewhat diagrammatic.[3] A, dorsal aspect; B, ventral aspect.

The anterior end is provided with a contractile suctorial tentacle which enables the ciliate to attach itself to the epithelial cells of the gills and palps of the host and to suck out their contents. The internal tubular canal continuous with the tentacle is directed at first dorsally and then ventrally and obliquely toward the right side of the body. It can usually be traced in fixed individuals stained with iron hematoxylin for about two-thirds the length of the body. Kofoid and Bush suggested only in the title of their paper that the form which they named *Parachaenia myae* was parasitic in *Mya arenaria,* but did not describe attachment of the ciliate to the epithelium. They found the ciliate in the pericardial cavity and excurrent siphon of the clams and apparently believed it to be unattached and to feed as a gymnostome, by producing a current in the medium by means of vigorous ciliary activity which carries food particles to the mouth. They stated that they observed a few instances of food taking, in which "debris containing bacteria enters the mouth and moves along the cytopharynx, forming little globules which continue back and aggregate in the large food vacuoles which distend the posterior part of the body." They stated further that "stained specimens show some vacuoles containing broken-up nuclear material similar to that of the epithelial cells which are removed when the fluid is taken from the clam." I have not observed any instances of ingestion of food such as that described by Kofoid and Bush, and although I admit it is perhaps possible for the ciliates to ingest food in this manner, I believe that they are primarily branchial parasites which feed by means of the suctorial tentacle.

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[2] Kofoid and Bush stated in their description of "*Parachaenia myae*" that the body of this ciliate is bilaterally compressed, the transverse diameter being about two-thirds the dorso-ventral diameter. Obviously their orientation of the form in question is not in agreement with the orientation assigned to it by Chatton and Lwoff, Raabe, and myself.

[3] All text-figures illustrating this paper are based on camera lucida drawings of individuals fixed in Schaudinn's fluid and impregnated with activated silver albumose (protargol).

The cilia of *A. pelseneeri* are disposed on the ventral, lateral, and dorso-lateral surfaces of the body in longitudinal rows originating at the anterior end. In all individuals which I examined carefully the number of ciliary rows was fourteen, but Raabe stated that in some specimens there are but thirteen rows. According to Raabe the ciliary system is composed of three separate complexes, the first consisting of eight or nine rows spiralling from the left side of the body toward the right and terminating progressively more posteriorly on the ventral surface, the second consisting of two approximately meridional rows situated on the central part of the ventral surface, and the third consisting of three rows spiralling from the right side of the body toward the left and terminating on the ventral surface. After studying a large number of the ciliates from *Mya arenaria* and *Macoma inconspicua* I cannot agree with Raabe on this matter. The ciliary rows appear collectively to form a single complex. There are usually five approximately equal rows about two-thirds the length of the body occupying the central portion of the ventral surface; these are bounded on the right by three progressively longer and more widely-spaced rows and on the left by six progressively longer and more widely-spaced rows. In some specimens the number of longer rows on the left side is greater than six, in which case the number of approximately equal and more or less meridional rows is proportionately decreased. Some of the outer rows on either side of the body, which originate on the lateral margins or on the dorsal surface, curve ventrally as they extend posteriorly, but the last two rows on the left side and the last row on the right side are typically dorso-lateral in position over their entire length. The outermost row on either side extends almost to the posterior tip of the body. Kofoid and Bush stated that the ciliary rows of *"Parachaenia myae"* may unite with one another, but I have never observed this to be the case, although in some seriously shrunken fixed individuals a few of the rows converge in such a way that they appear to be united.

In one of the illustrations accompanying the first of Raabe's papers in which there is a detailed discussion of *A. pelseneeri* (1934) the outermost ciliary row on the right side of the body is shown to originate as far anteriorly as the more central rows, while the outer three or four rows on the left side are shown to originate progressively more posteriorly. According to my own observations, however, the outermost row on the right side originates at about the same level as the last row on the left side. In all suitably impregnated individuals which I have studied the eighth row from the right side originates a little posterior to the level of origin of the adjacent ventral rows.

The cilia of *A. pelseneeri* are 8 μ to 10 μ in length. Those at the anterior end of the body are usually the more active and may be employed for thigmotactic attachment. Kofoid and Bush stated that the cilia of the "dorso-bilateral region" of *"Parachaenia myae"* are about 20 μ long near the anterior end, becoming somewhat shorter posteriorly; the cilia of the ventral surface, on the other hand, were said by them to be about one-half the length of those of the dorso-bilateral area. I have noted, however, no significant disparity between the lengths of the cilia of various parts of the ciliary system. When dissociated from the host the ciliate swims energetically, rotating on its longitudinal axis or swaying from side to side.

In the original description of *A. pelseneeri* given by Chatton and Lwoff reference is made to a "frange peristomienne" which they supposed corresponded to the peristomal fringe of cilia in species of *Ancistruma*. In his paper of 1934, Raabe described a short (approximately 13 μ long) row of basal granules lying in a dorsal anterior depression just above the anterior part of the internal tubular canal which he thought may represent the "frange peristomienne" described by Chatton and Lwoff. In his paper of 1938, however, Raabe stated that on certain of his preparations of this ciliate he could distinguish a row of basal granules such as he described in 1934, but did not refer to it as the peristomal fringe, and suggested that Chatton and Lwoff may have mistaken the stained outline of the internal tubular canal for a row of basal granules homologous with those of the peristomal fringe of ancistrumid ciliates. In my study of living, stained, and impregnated individuals of the ciliate I believe to be *A. pelseneeri* I have found no evidence whatever of a dorsal anterior depression or a row of basal granules such as that described by Raabe.

Kofoid and Bush described internal fibrillar structures, which they believed to represent elements of the neuromotor system, extending for a short distance posteriorly from an annular commissure ("cytostomal ring") around the "cytostome." One of the fibrils was said by them to pass along the internal tubular canal ("cytopharynx") to a slight thickening on the surface of the canal, then "towards the dorsal surface where it joins a relatively large granule which is closely associated with the mid-dorsal ciliary fibril." They stated further that "from points of the cytostomal ring on the ventral side, two fibrils are given off which soon unite and continue as a slender thread along the ventral surface of the cytopharynx." I have been unable to detect any structures in *A. pelseneeri* which might be construed as elements of a neuromotor system, but perhaps it is a siderophilic fibril-like structure of the type that Kofoid and Bush described that Raabe may have thought to represent a series of basal granules. The "cytostomal ring" around the "cytopharynx" was stated by Kofoid and Bush to be connected with the longitudinal ciliary rows,

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EXPLANATION OF PLATE I

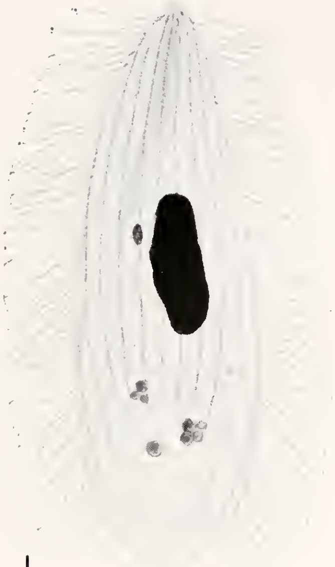
FIGURE 1. *Ancistrocoma pelseneeri* Chatton and Lwoff (from *Mya arenaria*). Ventral aspect. Heidenhain's "susa" fixative-iron hematoxylin. × 1,680.

FIGURE 2. *Ancistrocoma pelseneeri* Chatton and Lwoff (from *Macoma inconspicua*). Lateral aspect from left side, from life.

FIGURE 3. *Ancistrocoma dissimilis* sp. nov. Ventral aspect. Schaudinn's fixative-iron hematoxylin. × 1,680.

FIGURE 4. *Hypocomagalma pholadidis* sp. nov. Dorsal aspect. Schaudinn's fixative-iron hematoxylin. × 1,260.

FIGURE 5. *Hypocomagalma pholadidis* sp. nov. Ventral aspect. Schaudinn's fixative-iron hematoxylin. × 1,260.



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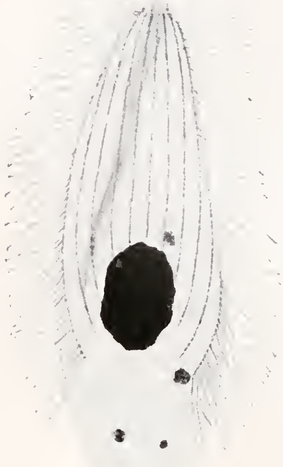

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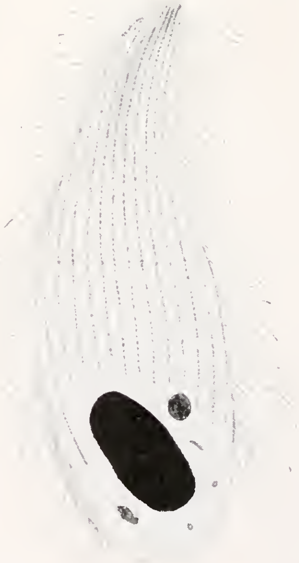

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Plate I

but I have not observed this to be the case in *A. pelseneeri*. As has been pointed out above, some of the rows do not originate as close to the base of the suctorial tentacle as others. It is possible that the structure referred to by Kofoid and Bush as the "cytostomal ring" represents the siderophilic anterior edge of the contracted suctorial tentacle.

The cytoplasm is colorless and contains numerous small refractile granules of a lipoid substance. In the posterior part of the body there are in addition to typical food vacuoles containing ingested fragments of epithelial cells one or more large vacuoles containing globular masses usually of a dense, homogeneous character. Raabe referred to this type of vacuole as "Konkrementenvacuole" and suggested that since he observed the internal tubular canal to terminate very near the "Konkrementenvacuole" the material within the vacuole may represent an accumulation of waste material which was not digested and absorbed as the ingested food material passed backward down the canal. It is quite true that these concrement vacuoles do not resemble the typical food vacuoles of most other ancistrocomid ciliates which I have studied. It would be interesting to determine whether or not digestion and absorption take place in the internal tubular canal, and how the material in the concrement vacuole, if it represents undigested wastes, is gotten rid of by the ciliate.

The macronucleus is usually sausage-shaped, rarely ovoid, and typically is situated dorsally near the middle of the body. In some fixed specimens stained with iron hematoxylin the chromatin appears to be distributed in irregular masses scattered through the macronuclear material; in other iron hematoxylin preparations and in most specimens stained by the Feulgen reaction the chromatin is aggregated into a dense reticulum enclosing vacuole-like clear spaces. In twenty individuals from *Mya arenaria* fixed in Schaudinn's fluid and stained by the Feulgen reaction the macronucleus ranged in length from 11 μ to 16 μ and in width from 4 μ to 7 μ.

The micronucleus is ovoid, fusiform, or sausage-shaped, and usually is seen to lie to the right of the macronucleus. In fixed and stained specimens the chromatin is ordinarily aggregated into granules. In twenty individuals from *Mya arenaria* fixed in Schaudinn's fluid and stained by the Feulgen reaction the micronucleus ranged in size from 1.2 μ by 3 μ to 2.1 μ by 3.2 μ.

Ancistrocoma pelseneeri is very common in *Mya arenaria* in all localities in San Francisco Bay where I have collected this mollusc. I have found it to be present, although usually in smaller numbers, also in *Cryptomya californica*, *Macoma inconspicua*, *M. nasuta*, and *M. irus* from several localities in San Francisco Bay, and in *Macoma secta* from Tomales Bay. It is peculiar that this ciliate was not recorded by Raabe from *Mya arenaria* at the marine biological station at Hel. Raabe listed *Sphenophyra dosiniae* Chatton and Lwoff, *Hypocomidium granum* Raabe, and a species of *Ancistruma* which he provisionally referred to *A. cyclidioides* (Issel), from *M. arenaria*. I have found *S. dosiniae* in a small percentage of *M. arenaria* and in a fairly large percentage of *Cryptomya californica* from San Francisco Bay. I have also found in *M. arenaria* the ciliate thought by Raabe to be *A. cyclidioides*, but not *Hypocomidium granum*.

Ancistrocoma pelseneeri Chatton and Lwoff (= *Parachaenia myae Kofoid and Bush*)

Diagnosis: Length 50 μ–83 μ (according to Kofoid and Bush 40 μ–100 μ), average about 62 μ; width 14 μ–20 μ, average about 16 μ; thickness 11 μ–16 μ, average about 12.5 μ. The ciliary rows are fourteen (according to Raabe thirteen or fourteen) in number and are distributed on the ventral, lateral, and dorso-lateral surfaces of the body. There are usually five approximately equal rows about two-thirds the length of the body on the ventral surface, bounded on the right by three progressively longer and more widely-spaced rows and on the left by six progressively longer and more widely-spaced rows. The outermost row on either side extends almost to the posterior tip of the body. The more central rows originate close to the base of the suctorial tentacle, while the several outer rows on either side originate progressively more posteriorly on the lateral margins and the dorsal surface. Some of these rows curve ventrally as they extend posteriorly, but the two outer rows on the left side and the outermost row on the right side are typically dorso-lateral in position over their entire length. The macronucleus is usually sausage-shaped. The micronucleus is ovoid, fusiform, or sausage-shaped. Parasitic on the epithelium of the gills and palps of *Macoma balthica* (L.) (Wimereux [Chatton and Lwoff] ; Hel [Raabe]) ; *Macoma inconspicua* Broderip and Sowerby, *Macoma nasuta* (Conrad), *Macoma irus* (Hanley), *Cryptomya californica* (Conrad) (San Francisco Bay, California) ; *Macoma secta* (Conrad) (Tomales Bay, California) ; *Mya arenaria* L. (Tomales Bay [Kofoid and Bush] ; San Francisco Bay).

ANCISTROCOMA DISSIMILIS SP. NOV.

(Figure 2; Plate I, Fig. 3)

The body is elongated, attenuated anteriorly, and somewhat flattened dorsoventrally. The ciliary system, to be described presently, is disposed for the most part on the incurved and slightly concave ventral surface. The body is widest and thickest in its posterior third and rounded posteriorly. Twenty living individuals taken at random ranged in length from 33 μ to 51 μ, in width from 10 μ to 14.5 μ, and in thickness from 8 μ to 12 μ, averaging about 44 μ by 13 μ by 10 μ.

The anterior end is provided with a contractile suctorial tentacle continuous with an internal tubular canal. The canal is directed at first dorsally and then ventrally and obliquely toward the right side of the body. In fixed specimens stained with iron hematoxylin it can usually be traced posteriorly for about one-half the length of the body.

The cilia of *A. dissimilis* are 7 μ to 8 μ in length and are disposed in longitudinal rows originating at the anterior end. The typical number of ciliary rows is eleven, but specimens with twelve rows are not uncommon, and I have seen some with fourteen rows. There are usually five approximately equal rows about three-fifths the length of the body occupying the central portion of the ventral surface; these are bounded on either side by three progressively longer rows, the outermost rows being three-fourths to four-fifths the length of the body. In specimens having twelve ciliary rows there are four longer rows on the left side instead of three; in specimens having fourteen rows there are four longer rows on the right side and five longer rows on the left. In some cases, particularly if the number of ciliary rows exceeds eleven, the five central rows are of unequal length, becoming progressively longer from right to left. One or two of the outer rows on either side originate on the lateral margin or the dorsal surface, usually a short distance posterior to the level of origin of the other rows. These rows curve ventrally and inward as they extend posteriorly, so that at least their distal portions are visible in ventral view.

The cytoplasm is colorless and contains numerous small refractile granules of a lipoid substance in addition to food inclusions. One or more larger food vacuoles are usually present in the posterior part of the body. The contractile vacuole lies near the middle of the body and opens to the exterior on the ventral surface.

The macronucleus is ovoid and situated dorsally near the middle of the body. In fixed and stained preparations the outline of the macronucleus is nearly always very irregular and the chromatin appears to be aggregated into a dense reticulum enclosing vacuole-like clear spaces of varying size. In twenty individuals fixed in Schaudinn's fluid and stained with iron hematoxylin the macronucleus ranged in length from 6.8 μ to 13.7 μ and in width from 5.4 μ to 7.2 μ.

The micronucleus is typically ovoid, rarely spherical, and commonly is situated a short distance anterior to or to one side of the macronucleus. In fixed and stained preparations the chromatin appears to be dispersed in granules of varying size. In twenty individuals fixed in Schaudinn's fluid and stained with iron hematoxylin the micronucleus ranged in size from 2.2 μ by 2.4 μ to 2.2 μ by 3.2 μ.

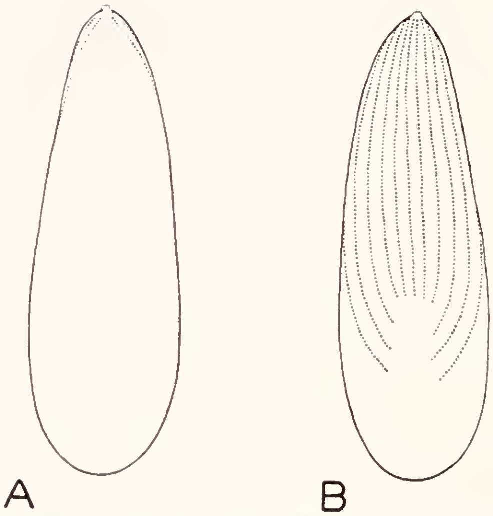

FIGURE 2. *Ancistrocoma dissimilis* sp. nov. Distribution of ciliary rows, somewhat diagrammatic. A, dorsal aspect; B, ventral aspect.

I found *Ancistrocoma dissimilis* to be present on the gills and palps of twenty-one of thirty-six specimens of *Pholadidea penita* which I examined from localities near Moss Beach, California. It is sometimes found in association with *Hypocomagalma pholadidis*. In some individuals of *P. penita* I have encountered a ciliate of the genus *Sphenophrya* which I hope to describe in a later paper and a species of *Boveria* which may also be new.

Ancistrocoma dissimilis sp. nov.

Diagnosis: Length 33 μ–51 μ, average about 44 μ; width 10 μ–14.5 μ, average about 13 μ; thickness 8 μ–12 μ, average about 10 μ. The ciliary rows are eleven to fourteen (typically eleven) in number and are distributed for the most part on the ventral surface and lateral margins of the body. Most of the rows originate on the ventral surface close to the base of the suctorial tentacle, while one or two outer rows on either side originate on the lateral margin or the dorsal surface and curve ventrally and inward as they extend posteriorly. There are usually five approximately equal rows about three-fifths the length of the body bounded on the right by three progressively longer rows and on the left by four progressively longer rows. The outermost row on either side is three-fourths to four-fifths the length of the body. The macronucleus is ovoid. The micronucleus is typically ovoid. Parasitic on the gills and palps of *Pholadidea penita* (Conrad) (Moss Beach, California). Syntypes are in the collection of the author.

HYPOCOMAGALMA PHOLADIDIS SP. NOV.

(Figure 3; Plate I, Figs. 4, 5)

The body is elongated, strongly attenuated anteriorly, and markedly asymmetrical. The anterior end is deflected toward the left and bent ventrally. The dorsoventral flattening characteristic of most ancistrocomid ciliates is not conspicuous in this species. As viewed from the posterior end the body appears in its middle and posterior portions to be almost as thick as wide. In its anterior third the body is nearly round in cross section. Most fixed specimens are considerably distorted and compressed in such a way that they appear to be widest near the middle. Twenty living individuals taken at random ranged in length from 63 μ to 89 μ, in width from 18 μ to 25 μ, and in thickness from 16 μ to 21 μ, averaging about 76 μ by 22 μ by 19 μ.

The anterior end is provided with a contractile suctorial tentacle continuous with an internal tubular canal. The canal can usually be traced in fixed specimens stained with iron hematoxylin down the middle of the attenuated anterior part of the body and then obliquely toward the right side. I have not succeeded in demonstrating the course of the canal beyond the anterior one-third of the body.

The cilia of *Hypocomagalma pholadidis* are approximately 9 μ to 10 μ in length. The ciliary system consists of twenty-four or twenty-five longitudinal rows. The body is almost completely invested by cilia except for a cilia-free "cap" at the posterior end. Two rows on the right side of the body usually appear to be set apart from the others, but in some specimens the spacing between these rows and the adjacent rows on either side is not significantly wider than the spacing between some of the other rows. Perhaps these two rows are homologous with the one or two rows constituting the right ciliary complex of *Crebricoma carinata* (Raabe), *Insignicoma venusta* Kozloff, and species of *Hypocomides*. They originate near the base of the suctorial tentacle on the right margin or the dorsal surface close to the right margin and curve ventrally and to the left as they extend backward. The outer row, as seen in ventral view, is the longer and extends almost to the posterior end of the body. The inner row terminates a short distance more anteriorly than the outer row, but is conspicuously longer than the first of the next series of rows, which usually is about two-thirds the length of the body. The first eight to ten rows to the left of the two longer rows all originate at about the same level on the ventral surface close to the base of the suctorial tentacle. The remaining rows, which are disposed along the left margin of the body and on the dorsal surface, originate progressively more posteriorly. The tenth or eleventh row of this complex is usually the longest, although some of the shorter rows on the dorsal surface may terminate more posteriorly. The last ciliary row on the right side of the dorsal surface is always the shortest row, originating at a point about one-third the distance from the anterior end of the body to the posterior end and terminating at a point about three-fourths or four-fifths the distance from the anterior end to the posterior end.

The cytoplasm is colorless and contains numerous small refractile granules of a lipoid substance in addition to food inclusions. One or more larger food vacuoles containing fragments of cells from the epithelial tissues of the gills or palps of the host are usually evident in the posterior part of the body. The contractile vacuole, when single, is located near the middle of the body and opens to the exterior on the ventral surface. In a larger percentage of the living specimens of *H. pholadidis* which I examined there were two or more contractile vacuoles scattered through the body which emptied their contents to the exterior on the ventral surface. In a large percentage of the living specimens of *H. pholadidis* which I examined there were two or more contractile vacuoles scattered through the body which emptied their contents to the exterior independently of one another. Jarocki and Raabe (1932) reported that in *H. dreissenae* the contractile vacuole was sometimes single, but that in some specimens there were several smaller ones.

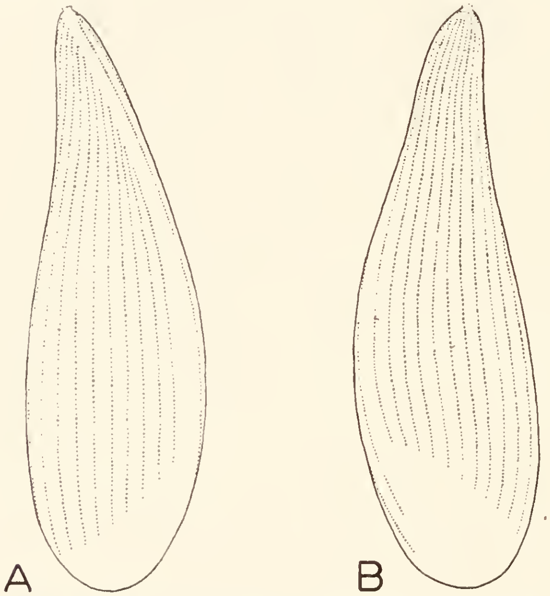

FIGURE 3. *Hypocomagalma pholadidis* sp. nov. Distribution of ciliary rows, somewhat diagrammatic. A, dorsal aspect; B, ventral aspect.

The macronucleus typically is sausage-shaped and lies in the posterior third of the body, its longitudinal axis placed obliquely to the longitudinal axis of the body. In light iron hematoxylin preparations and in specimens stained by the Feulgen reaction the chromatin of the macronucleus appears to be aggregated into a dense reticulum enclosing vacuole-like spaces which frequently contain globular masses of deeply-staining material. In ten individuals fixed in Schaudinn's fluid and stained by the Feulgen reaction the macronucleus ranged in length from 12.5 μ to 20 μ and in width from 5 μ to 8.9 μ.

The micronucleus is spherical and usually is situated a short distance anterior to or to one side of the macronucleus. In most fixed and stained preparations the chromatin appears to be homogeneous, although in some the chromatin appears to be in part aggregated into granules or peripheral strands. In ten individuals fixed in Schaudinn's fluid and stained by the Feulgen reaction the diameter of the micronucleus ranged from 2.4 μ to 3.3 μ.

I found *Hypocomagalma pholadidis* to be present on the gills and palps of twenty-eight of thirty-six specimens of *Pholadidea penita* which I examined from localities near Moss Beach, California. When the ciliate is dissociated from the host it swims erratically, usually rotating on its longitudinal axis and tracing wide arcs with its attenuated anterior end. The cilia of the anterior half of the body are more active than those of the posterior half and are sometimes observed to beat metachronously. The ventral cilia near the base of the suctorial tentacle are markedly thigmotactic.

Hypocomagalma pholadidis sp. nov.

Diagnosis: Length 63 μ–89 μ, average about 76 μ; width 18 μ–25 μ, average about 22 μ; thickness 16 μ–21 μ, average about 19 μ. The anterior end of the body is attenuated, conspicuously deflected toward the left, and bent ventrally. The ciliary system consists of twenty-four or twenty-five rows. Two long rows on the right side of the body appear in most specimens to be set apart from the remaining rows; these two rows originate near the base of the suctorial tentacle and extend almost to the posterior end of the body. The first eight to ten rows to the left of these two longer rows originate at about the same level on the ventral surface, while the remaining rows, disposed along the left lateral margin and the dorsal surface, originate progressively more posteriorly. The contractile vacuole may be single or represented by several independent vacuoles opening to the exterior on the ventral surface. The macronucleus is sausage-shaped. The micronucleus is spherical. Parasitic on the epithelium of the gills and palps of *Pholadidea penita* (Conrad) (Moss Beach, California). Syntypes are in the collection of the author.

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STUDIES ON CILIATES OF THE FAMILY ANCISTROCOMIDAE CHATTON AND LWOFF (ORDER HOLOTRICHA, SUBORDER THIGMOTRICHA)

IV. HETEROCINETA JANICKII JAROCKI, HETEROCINETA GONIOBASIDIS SP. NOV., HETEROCINETA FLUMINICOLAE SP. NOV., AND ENERTHECOMA PROPERANS JAROCKI

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Introduction

The genus *Heterocineta* was established by Mavrodiadi (1923) for a ciliate which he named *Heterocineta anodontae*, and which he had formerly believed to represent a gregariniform stage in the development of *Conchophthirus anodontae* (Ehrenberg). Unaware of the fact that Mavrodiadi had abandoned his earlier conception and applied the name *Heterocineta anodontae* to this ciliate, Jarocki and Raabe (1932) described the same species, from *Anodonta cygnea* (L.) and *Unio pictorum* L., as *Hypocomatophora unionidarum*. Jarocki later (1934) pointed out that *Hypocomatophora unionidarum* was a synonym of *Heterocineta anodontae*.

In his papers of 1934 and 1935 Jarocki described seven additional species of the genus *Heterocineta* ectoparasitic on fresh water gastropods: *H. janickii*, from *Physa fontinalis* (L.); *H. lwoffi*, from *Viviparus fasciatus* Müller; *H. chattoni*, from *Radix ovata* (Drap.); *H. krzysiki*, from *Bithynia tentaculata* (L.); *H. maziarskii*, from *Coretus corneus* (L.); *H. turi*, from *Tropidiscus planorbis* (L.) and *Spiralina vortex* (L.); and *H. siedleckii*, from *Acroloxus lacustris* (L.). In 1945 I described as *Heterocineta phoronopsidis* a ciliate from the tentacles of *Phoronopsis viridis* Hilton. This species is the only representative of the genus thus far described which is not a parasite of fresh water molluscs or of the annelid commensal *Chaetogaster limnaci* von Baer when the latter is associated with infected snails.

On the fresh water prosobranch snails *Goniobasis plicifera silicula* (Gould) and *Fluminicola virens* (Lea) I have found two new species of *Heterocineta* which will be described herein as *H. goniobasidis* sp. nov. and *H. fluminicolae* sp. nov. I have also studied a species of *Heterocineta* from *Physa cooperi* Tryon which agrees with the original description of *H. janickii*. It seems advisable, for comparative purposes, and in view of the fact that Jarocki's description of *H. janickii* is not accompanied by illustrations, to include an account of the morphology of this form in the present paper.

The genus *Enerthecoma* was proposed by Jarocki (1935) for a single species, *E. properans*, parasitic on the gills of *Viviparus fasciatus*. Although the original description of this species is quite adequate, it is not supplemented by illustrations, and the second installment of Jarocki's "Studies on ciliates from fresh-water molluscs," in which figures of *E. properans* and several other ciliates were to be published, has not come to my attention. A ciliate which I have found to infest *Viviparus malleatus* (Reeve) apparently is identical with *E. properans*. This ciliate will be described and illustrated here.

HETEROCINETA JANICKII JAROCKI

(Figure 1; Plate I, Fig. 1)

The body is elongated and flattened dorso-ventrally. The anterior end is attenuated, bent ventrally, and deflected slightly toward the left. The anterior one-half of the left margin is not quite so rounded as the right margin and typically is nearly straight or weakly indented. The body is widest a short distance behind the middle and rounded posteriorly. The ciliary system, to be described presently, is disposed on a shallow concavity occupying the anterior two-thirds of the ventral surface; the dorsal surface and that part of the ventral surface posterior to the ciliary area are convex. Twenty living individuals from *Physa cooperi* ranged in length from 25 μ to 32 μ, in width from 12 μ to 15 μ, and in thickness from 10 μ to 12 μ, averaging about 30 μ by 14 μ by 11 μ. The specimens of *H. janickii* from *Physa fontinalis* which were studied by Jarocki ranged in length from 23 μ to 32 μ, in width from 12 μ to 17 μ, and in thickness from 10 μ to 13 μ.

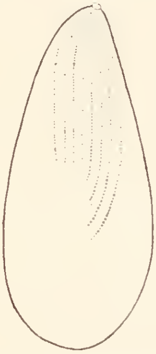

FIGURE 1. *Heterocineta janickii* Jarocki. Distribution of ciliary rows, somewhat diagrammatic.[1] Ventral aspect.

The anterior end of the body is provided with a short contractile suctorial tentacle which enables the ciliate to attach itself to the epithelial cells of the host and to feed upon their contents. When fully extended the tentacle is about 3 μ to 4 μ (according to Jarocki about 4.5 μ) in length. The internal tubular canal continuous with the tentacle is directed at first dorsally and then ventrally and obliquely toward the right side, and in specimens stained with iron hematoxylin can usually be traced for about one-half the length of the body.

The ciliary system consists of eight longitudinal rows originating close to the base of the suctorial tentacle. The first four rows from the right side are approximately one-half the length of the body. The remaining four rows become increasingly longer and terminate one behind the other a little to the left of the midline.

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[1] The text figures illustrating this paper are based on camera lucida drawings of specimens impregnated with silver nitrate by Klein's method.

The longest row is about two-thirds the length of the body. The cilia are about 6 μ to 7 μ (according to Jarocki about 5 μ to 7 μ) in length. While attached to the skin of the host the parasites are as a rule almost immobile, their cilia exhibiting only a feeble motion. When dissociated from the host *Heterocineta janickii* swims sluggishly, usually rotating on its longitudinal axis and tracing wide arcs with its attenuated anterior end.

The cytoplasm is colorless and contains numerous small refractile granules in addition to food inclusions. One or more large food vacuoles are present in the posterior part of the body behind the macronucleus. The contractile vacuole is situated near the middle of the body and opens to the exterior on the ventral surface. I have observed no permanent opening in the pellicle.

The macronucleus is typically sausage-shaped and is located near the middle of the body or somewhat posterior to the middle. As seen in dorsal or ventral view the longitudinal axis of the macronucleus is placed obliquely to the longitudinal axis of the body. As seen in lateral view, the anterior end of the macronucleus is directed dorsally, while the posterior end is directed ventrally. In fixed and stained preparations the chromatin appears to be more or less homogeneous. In ten individuals fixed in Schaudinn's fluid and stained by the Feulgen reaction the macronucleus ranged in length from 7 μ to 11 μ and in width from 4 μ to 5 μ.

The micronucleus is ovoid or spherical and is situated near the dorsal surface anterior to or to one side of the macronucleus. In most fixed and stained specimens the chromatin is homogeneous, although in some it appears to be concentrated in peripheral granules. In ten individuals fixed in Schaudinn's fluid and stained with iron hematoxylin the size of the micronucleus ranged from 1.4 μ by 1.4 μ to 1.6 μ by 2 μ.

Heterocineta janickii was present in very small numbers on the tentacles, mantle, and margins of the foot of most of the specimens of *Physa cooperi* which I collected in a stream near Mt. Eden, California. The degree of infestation increased rapidly on snails kept in laboratory aquaria for a period of six weeks.

Heterocineta janickii Jarocki

Diagnosis: Length 25 μ–32 μ (according to Jarocki 23 μ–32 μ), average about 30 μ; width 12 μ–15 μ (according to Jarocki 12 μ–17 μ), average about 14 μ; thickness 10 μ–12 μ (according to Jarocki 10 μ–13 μ), average about 11 μ. The ciliary system consists of eight rows originating close to the base of the suctorial tentacle. The first four rows from the right are about one-half the length of the body, while the remaining four rows become progressively longer and terminate one behind the other a little to the left of the midline. The longest row is about two-thirds the length of the body. Parasitic on the epithelium of the tentacles, mantle, and foot of *Physa fontinalis* (L.) (Warsaw [Jarocki]) and *Physa cooperi* Tryon (Mt. Eden, California).

HETEROCINETA GONIOBASIDIS SP. NOV.

(Figure 2; Plate I, Figs. 2, 3)

The body is elongated and flattened dorso-ventrally. The anterior end is attenuated, bent ventrally, and deflected slightly toward the left. The anterior one-half of the left margin is not so rounded as the right margin and typically is nearly straight or weakly indented. The body is widest at the middle or a short distance anterior to the middle. The ciliary system is disposed on a shallow concavity occupying the anterior two-thirds of the ventral surface; the dorsal surface and that part of the ventral surface posterior to the ciliary area are convex. Twenty-five living specimens taken at random ranged in length from 36 μ to 48 μ, in width from 15 μ to 20 μ, and in thickness from 11 μ to 14 μ, averaging about 43 μ by 18 μ by 13 μ.

The anterior end is provided with a contractile suctorial tentacle continuous with an internal tubular canal. The nature of the canal is very similar to that of other members of the genus. It is directed at first dorsally and then ventrally and obliquely toward the right side of the body. It can be traced in most fixed specimens stained with iron hematoxylin for about one-half to two-thirds of the length of the body.

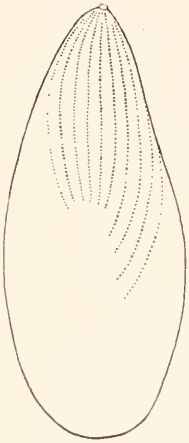

FIGURE 2. *Heterocineta goniobasidis* sp. nov. Distribution of ciliary rows, somewhat diagrammatic. Ventral aspect.

The cilia of *H. goniobasidis* are about 9 μ long. Those of the anterior part of the ciliary system are markedly thigmotactic. The ciliary system consists of ten longitudinal rows. The first six rows are approximately the same length, being about one-half the length of the body, although on careful examination the first row is seen to originate some distance posterior to the level of origin of the other five rows. The seventh, eighth, ninth, and tenth rows originate progressively more posteriorly and become increasingly longer, terminating one behind the other a little to the left of the midline. The longest row is two-thirds to three-fourths the length of the body. The last one or two rows usually originate on the left margin and curve ventrally as they extend backward. The cilia of the distal portions of the longer rows are nearly always practically motionless and directed posteriorly. When dissociated from the host the ciliate swims sluggishly and erratically, rotating on its longitudinal axis.

The cytoplasm is colorless and contains numerous refractile granules of a lipoid substance in addition to food inclusions. There are usually one or two large food vacuoles in the posterior part of the body behind the macronucleus. The contractile vacuole is central and opens to the exterior on the ventral surface.

The macronucleus is situated in the middle portion of the body. It is elongated and typically somewhat narrower at its anterior end than at its posterior end. As seen in dorsal or ventral aspect, the longitudinal axis of the macronucleus is placed obliquely to the longitudinal axis of the body. As seen in lateral view, the anterior end of the macronucleus is directed dorsally, while the posterior end is directed ventrally. In ten individuals fixed in Schaudinn's fluid and stained with iron hematoxylin the macronucleus ranged in length from 10 μ to 13.5 μ and in width from 4 μ to 5.5 μ.

The spherical or ovoid micronucleus is very difficult to distinguish in the living ciliates. It is usually situated near the dorsal surface a short distance anterior to the macronucleus. In fixed and stained preparations the micronucleus is vesicular, the chromatin being concentrated along the periphery. In ten individuals fixed in Schaudinn's fluid and stained with iron hematoxylin the micronucleus ranged in size from 1.2 μ by 1.5 μ to 1.5 μ by 1.7 μ.

Heterocineta goniobasidis was found to be present on the epithelium of the gills and mantle of a small percentage of the specimens of *Goniobasis plicifera silicula* which I collected in Crystal Springs Creek, in Portland, Oregon. The degree of infestation on freshly collected snails was very low, but increased during the four weeks the specimens were kept in laboratory aquaria.

Heterocineta goniobasidis sp. nov.

Diagnosis: Length 36 μ–48 μ, average about 43 μ; width 15 μ–20 μ, average about 18 μ; thickness 11 μ–14 μ, average about 13 μ. The ciliary system is composed of ten rows. The first six rows from the right side are about one-half the length of the body and, with the exception of the first row, originate close to the base of the suctorial tentacle. The remaining rows originate progressively more posteriorly and become increasingly longer, terminating one behind the other a little to the left of the midline. The longest row is two-thirds to three-fourths the length of the body. Parasitic on the gills and mantle of *Goniobasis plicifera silicula* (Gould) (Portland, Oregon). Syntypes are in the collection of the author.

HETEROCINETA FLUMINICOLAE SP. NOV.

(Figure 3; Plate I, Fig. 4)

The body is elongated and flattened dorso-ventrally. The anterior end is attenuated, bent ventrally, and deflected slightly toward the left. The anterior part

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EXPLANATION OF PLATE I

All figures except Figure 2 have been prepared with the aid of a camera lucida.

FIGURE 1. *Heterocineta janickii* Jarocki. Ventral aspect. Schaudinn's fixative-iron hematoxylin. × 1,720.

FIGURE 2. *Heterocineta goniobasidis* sp. nov. Lateral aspect from left side, from life.

FIGURE 3. *Heterocineta goniobasidis* sp. nov. Ventral aspect. Schaudinn's fixative-iron hematoxylin. × 1,720.

FIGURE 4. *Heterocineta fluminicolae* sp. nov. Ventral aspect. Schaudinn's fixative-iron hematoxylin. × 1,720.

FIGURE 5. *Enerthecoma properans* Jarocki. Macro- and micronuclei from three specimens. Schaudinn's fixative-Feulgen reaction. × 1,720.

FIGURE 6. *Enerthecoma properans* Jarocki. Ventral aspect. Schaudinn's fixative-iron hematoxylin. × 1,720.

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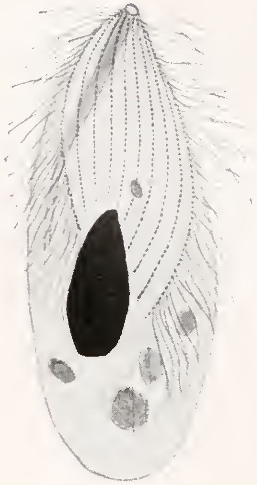

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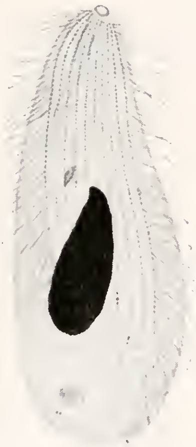

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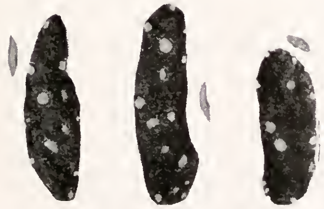

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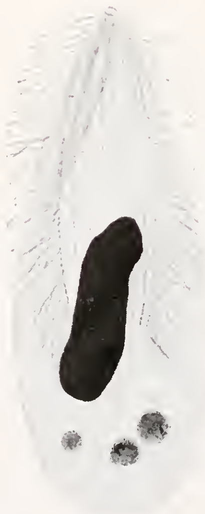

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Plate I

of the left margin is not so rounded as the right margin and typically is weakly indented. The body is widest a short distance behind the middle and rounded posteriorly. The ciliary system is disposed on a shallow concavity occupying the major portion of the ventral surface; the dorsal surface and that part of the ventral surface posterior to the ciliary area are convex. Twenty-five living individuals taken at random ranged in length from 30 μ to 36 μ, in width from 13 μ to 17 μ, and in thickness from 10 μ to 12 μ, averaging about 33 μ by 15 μ by 11 μ.

The anterior end is provided with a contractile suctorial tentacle continuous with an internal tubular canal. The canal is directed at first ventrally and then obliquely toward the right side of the body. It can be traced in most fixed specimens stained with iron hematoxylin for about one-half the length of the body.

The cilia of *H. fluminicolae* are about 6 μ or 7 μ long. Those of the anterior part of the ciliary system are strongly thigmotactic. The ciliary system consists of ten longitudinal rows. The first row on the right side of the ciliary complex originates close to the base of the suctorial tentacle; each of the remaining rows originates progressively more posteriorly. The first six rows from the right side are approximately the same length, being about two-thirds the length of the body. The last four rows become increasingly longer and incurved in such a way that they terminate one behind the other not far to the left of the midline. The longest row usually extends almost to the posterior end of the body. The cilia of the distal portions of these longer rows are usually directed posteriorly. When the ciliate is dissociated from the host it swims erratically, rotating on its longitudinal axis and tracing wide arcs with its anterior end.

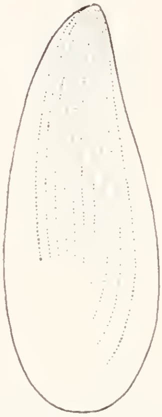

FIGURE 3. *Heterocincta fluminicolae* sp. nov. Distribution of ciliary rows, somewhat diagrammatic. Ventral aspect.

The cytoplasm is colorless and contains numerous small refractile granules of a lipoid substance in addition to food inclusions. One or more large food vacuoles are usually present in the posterior part of the body behind the macronucleus. The contractile vacuole is central and opens to the exterior on the ventral surface. I have not observed a permanent opening in the pellicle.

The sausage-shaped macronucleus is situated dorsally a short distance behind the middle of the body with its longitudinal axis placed obliquely to the longitudinal axis of the body. In fixed and stained preparations the chromatin appears to be more or less homogeneous. In ten individuals fixed in Schaudinn's fluid and stained with iron hematoxylin the macronucleus ranged in length from 7.4 μ to 10 μ and in width from 3.9 μ to 4.4 μ.

The micronucleus is round, fusiform, or ovoid, and is usually placed dorsally near the middle of the body anterior to or to one side of the macronucleus. In fixed and stained specimens the chromatin is seen to be concentrated primarily along the periphery. In ten individuals fixed in Schaudinn's fluid and stained with iron hematoxylin the micronucleus ranged in size from 1.5 μ by 1.2 μ to 1.7 μ by 1.5 μ.

Heterocineta fluminicolae was present in small numbers on the epithelium of the gills and the edge of the mantle of nearly all specimens of *Fluminicola virens* which I collected in Crystal Springs Creek in Portland, Oregon.

Heterocineta fluminicolae sp. nov.

Diagnosis: Length 30 μ–36 μ, average about 33 μ; width 13 μ–17 μ, average about 15 μ; thickness 10 μ–12 μ, average about 11 μ. The ciliary system is composed of ten rows originating progressively more posteriorly from the right side to the left. The first six rows from the right side are about two-thirds the length of the body. The remaining four rows become increasingly longer and terminate one behind the other a little to the left of the midline. The longest row extends almost to the posterior end of the body. Parasitic on the gills and mantle of *Fluminicola virens* (Lea) (Portland, Oregon). Syntypes are in the collection of the author.

ENERTHECOMA PROPERANS JAROCKI

(Figure 4; Plate I, Figs. 5, 6)

The body is elongated, nearly symmetrical as seen in dorsal or ventral view, attenuated anteriorly, and flattened dorso-ventrally. The anterior end is bent ventrally and deflected inconspicuously toward the left. The ciliary system is disposed on a narrow, relatively flat area occupying the anterior two-thirds of the ventral surface; the dorsal surface and that part of the ventral surface posterior to the ciliary area are convex. The body is widest at a point about two-thirds the distance from the anterior end to the posterior end. Twenty-five living individuals taken at random from *Viviparus malleatus* ranged in length from 32 μ to 56 μ, in width from 13 μ to 21 μ, and in thickness from 10 μ to 13 μ, averaging about 44 μ by 18 μ by 11.5 μ. Specimens from *Viviparus fasciatus* which were measured by Jarocki ranged in length from 33 μ to 60 μ, in width from 15 μ to 22 μ, and in thickness from 10 μ to 13 μ.

The contractile suctorial tentacle is continuous with an internal tubular canal which is directed at first dorsally and then ventrally and obliquely toward the right side of the body. In specimens stained with iron hematoxylin the canal can usually be traced for about two-thirds or three-fourths the length of the body.

The ciliary system is composed of eight approximately equal rows about two-thirds the length of the body. These rows originate close to the base of the suctorial tentacle. The first five rows from the right side are usually a little more widely spaced than the last three rows. This was noted also by Jarocki. who stated that the ciliary system was separated into two complexes by an "inconsiderable eminence stretching from the base of the tentacle to the end of the system," which segregated the five rows on the right from the three rows on the left. This eminence was evident on many of the living specimens which I examined but is never conspicuous. The cilia of *E. properans* are about 9 μ in length and exhibit a feeble undulatory motion while the parasites are attached to the epithelium of the gills of the host. When dissociated from the host the ciliates swim slow and erratically, usually rotating on their longitudinal axes.

The cytoplasm is colorless and contains numerous small refractile granules of a lipoid substance in addition to food inclusions. One or more larger food vacuoles are usually present in the posterior part of the body. The contractile vacuole is situated a short distance behind the middle of the body and opens to the exterior on the ventral surface. I have not detected a permanent opening in the pellicle.

The macronucleus is typically sausage-shaped and is situated in the posterior half of the body with its longitudinal axis placed obliquely to the longitudinal axis of the body. In specimens stained with iron hematoxylin the chromatin appears to be more or less homogeneous, but in preparations stained by the Feulgen reaction it appears to be organized into a dense reticulum enclosing vacuole-like clear spaces of varying size. In ten individuals fixed in Schaudinn's fluid and stained by the Feulgen reaction the macronucleus ranged in length from 10 μ to 19 μ and in width from 4 μ to 7 μ.

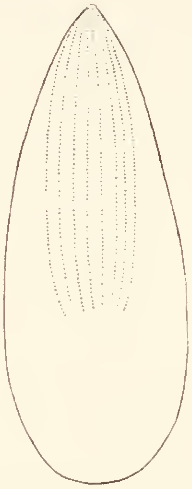

FIGURE 4. *Enerthecoma properans* Jarocki. Distribution of ciliary rows, somewhat diagrammatic. Ventral aspect.

The micronucleus is situated anterior to or to one side of the macronucleus. In most of the individuals of *E. properans* which I examined, the micronucleus is elongated and more or less fusiform. I have observed very few specimens to have a round micronucleus such as that described by Jarocki. The micronucleus does not stain readily with iron hematoxylin and it is possible that Jarocki may have mistaken food inclusions for micronuclei. In specimens stained by the Feulgen reaction the chromatin of the micronucleus appears to be concentrated in peripheral granules or strands. In ten individuals fixed in Schaudinn's solution and stained by the Feulgen reaction the micronucleus ranged in size from 0.8 μ by 2.3 μ to 1 μ by 3.8 μ.

Enerthecoma properans was abundant on the gills of nearly all specimens of *Viviparus malleatus* which I collected in Stow Lake, San Francisco, California, and in Evans Lake, Riverside, California. It is undoubtedly a common parasite of this introduced snail wherever the latter has become established.

Enerthecoma properans Jarocki

Diagnosis: Length 32 μ–56 μ (according to Jarocki 33 μ–60 μ), average about 44μ; width 13 μ–21 μ (according to Jarocki 15 μ–22 μ), average about 18 μ; thickness 10 μ–13 μ, average about 11.5 μ. The ciliary system is composed of eight approximately equal rows about two-thirds the length of the body which originate close to the base of the suctorial tentacle and occupy a narrow, relatively flat area on the ventral surface. The first five rows from the right are more widely-spaced than the remaining three rows, and in living specimens appear to be segregated from the latter by an inconspicuous longitudinal eminence. The macronucleus is elongated; the micronucleus is typically elongated and more or less fusiform (according to Jarocki, spherical). Parasitic on the gills of *Viviparus fasciatus* Müller (Warsaw [Jarocki]) and *Viviparus malleatus* (Reeve) (San Francisco, California; Riverside, California).

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PROGRAM AND ABSTRACTS OF SCIENTIFIC PAPERS PRESENTED AT THE MARINE BIOLOGICAL LABORATORY, SUMMER OF 1946

JULY 9

Dr. J. E. Kindred. No abstract submitted.

The cyanide sensitivity of the unfertilized sea urchin egg. W. A. Robbie.

Reinvestigation of the cyanide sensitivity of unfertilized eggs of *Arbacia punctulata*, using recently devised methods for the control of HCN concentration in manometric experiments, showed that there was a definite inhibition of respiration. The respiration is depressed by concentrations of HCN as low as 10^{-5} M., and for a four-hour period in 10^{-4} M. it is only 40 per cent of the control value. There is complete inhibition for the first hour or more. In 4 per cent O_2-96 per cent N_2 mixture there is no depression of the respiration of the control egg, but on the addition of 10^{-4} M. HCN the oxygen consumption is reduced, for a four-hour exposure, to 20 per cent of the control level.

At concentrations of cyanide higher than 10^{-4} M. there is apparently a stimulation in oxygen uptake. This is increased with high and reduced with low oxygen tensions. It is possibly associated with oxidations proceeding through a cyanide-hemin system, or with the metabolism of a carbohydrate intermediate catalyzed by HCN.

Inhibition of fertilization in sea urchins by means of univalent antibodies vs. antifertilizin. Albert Tyler.

In order to obtain further information as to the role of the specific interacting substance of eggs and sperm in fertilization, antisera were prepared against them by immunization of rabbits, and the antibodies tested for their ability to interfere with fertilization. The present report concerns tests with antibodies prepared against purified anifertilizin derived from sperm of the sea urchin *Lytechinus pictus* and the gephyrean worm *Urechis caupo*. The antisera agglutinate the species sperm to high titer, but cannot be used directly to test for specific action on fertilization, since the mechanical effect of tying up the sperm would itself constitute a block to fertilization. However, by a previously described method, namely photo-oxidation, antibodies can be converted into a non-agglutinating form, termed "univalent." This treatment was, therefore, applied to the anti-antifertilizin sera and the "univalent" antibodies thus obtained were tested for possible action on the ability of the sperm to fertilize eggs of the same species. The results showed a considerable reduction in fertilizing power of the sperm, ranging in different tests from 32-fold to greater than 128-fold. At the same time, the motility of the treated sperm was found to be quite as high as the controls.

JULY 16

Intermediate steps in the visual cycle. A. F. Bliss.

The primary functions of a visual pigment are absorption of radiant energy and its transfer to the stimulatory mechanism of the visual cell. At present four such pigments are known: rhodopsin and porphyropsin, the photosensitive pigments of vertebrate night vision; iodopsin, the corresponding pigment of daylight vision; and cephalopsin, the photostable red pigment of cephalopods and probably other invertebrates (*J. Gen. Physiol.*, 1943). Instability in the light has generally been accepted as a diagnostic test for a visual pigment. The existence of a light-stable visual pigment in the squid however throws doubt on the validity of this assumption.

The bleaching by light of vertebrate visual pigments is nevertheless an interesting and complex process which has not been adequately analyzed into its component steps. The first known product of bleaching visual purple is a thermally unstable complex lipid, called Transient Orange by Lythgoe and Provisual Red by Krause. Its sluggish reaction to base and temperature near 0° C. suggest that it is the acid tautomer of the first relatively stable product of bleaching, appropriately named Indicator Yellow by Lythgoe. Acid Indicator Yellow is a red lipid, becoming reversibly decolorized in base, and irreversibly converted in chloroform to the greenish yellow carotenoid retinene, extracted by Wald from freshly bleached frog retinas. If bleached retinas are allowed to stand an hour before extraction, retinene is no longer found. Instead an equivalent amount of vitamin A is extracted. Retinene, however, is not the precursor to vitamin A, but is due to the irreversible side reaction described above. In the normal retina and in fresh neutral solution Indicator Yellow forms vitamin A under the influence of a labile protein, the reaction being presumbaly enzymatic in nature. In the dark, dissolved rhodopsin is reformed in part from Indicator Yellow. In the living animal the vitamin A released by bleaching is reincorporated into rhodopsin by unknown means.

MR. W. H. PRICE. No abstract submitted.

*The dependence of the resting potential of nerve on potassium, calcium, and hydrogen ions.** ABRAHAM M. SHANES.

On the basis of new as well as recent experimental results it now appears possible to describe a specific mechanism necessary and sufficient to account for the relationships between the resting potential and metabolic processes in frog nerve. Cellular hydrogen ion production accompanied by an exchange with extracellular potassium ions is apparently involved; under the conditions of study this process contributes about 50 per cent of the total resting potential. Calcium reduces the rate of ionic exchange, an effect of possible importance in the energy expenditure necessary to maintain concentration gradients and associated potentials.

The evidence consists of demonstrating first that hydrogen ions from (1) CO_2 produced by nerve, (2) CO_2 applied to nerve, and (3) lactic acid and possibly other sources of acid within the fibers are directly concerned with the production and maintenance of the potentials. This has been possible chiefly with the aid of inhibitors of carbonic anhydrase—sulfanilamide and thiophene-2-sulfonamide—by means of which the role of hydrogen ions can be followed during anoxia, upon return to oxygen following anoxia, and to some extent during relatively normal aerobic conditions. The changes of potential associated with the application of CO_2-O_2 mixtures and related experiments show that the effectiveness of the hydrogen ions is dependent on the ionic gradients established.

The involvement of extracellular potassium is demonstrated by the suppression in its absence of the changes in potential normally produced by CO_2. This effect is used to show, further, that the potassium in the extracellular spaces is reduced by the rapid large increase in potential induced by the return of the anoxic nerves to oxygen. A small secondary decline of potential which follows the rise in CO_2 and which is independent of extracellular buffering is also dependent on extracellular potassium, which suggests that the dense connective tissue or the other sheathing materials of nerve are interfering with the potassium exchange between the extracellular space immediately adjacent to the fibers and that more remote.

Calcium slows the rate of potential rise upon application of CO_2 and this effect is directly related to calcium concentration; in view of the above results and available evidence, this is interpreted as an effect on ionic exchange. At lower concentrations calcium depresses to almost the same degree the potential changes in response to oxygen following anoxia and to CO_2; higher calcium concentrations, known to suppress the metabolic processes, exert a more marked effect on the former.

These results therefore focus attention on factors important in the production and modification of the resting potential. The action of any agent on the potential must be considered from several possible standpoints: (1) inhibition or activation of metabolism or of carbonic anhydrase, (2) production of hydrogen ions, (3) production of a membrane diffusion potential, (4) modification of equilibrium or membrane diffusion potentials. The experimental procedures which have been applied provide means of distinguishing these possibilities. In view of the conclusions reached, these methods should also prove useful in studies of the "potassium pump" and of the biochemical processes concerned with CO_2 production and fixation in relatively intact cells, both are problems of considerable interest at the present time.

* Aided by a grant from the Penrose Fund of the American Philosophical Society.

Dr. J T. Bonner. No abstract submitted.

July 23

Oxidation-reduction studies as a clue to the mechanism of fertilization of marine eggs. Matilda M. Brooks.

Eggs, sperm, and larvae at stages up to pluteus of three marine animals (*Arbacia punctulata, Asterias Forbesii* and *Chaetopterus pergementaceus*) were measured for E_h and Ph. The egg, sperm or larvae were centrifuged and 1 cc. of the mass used for measurement in a glass vessel in the Coleman electrometer. It was found that there is a definite correlation between the rate of O_2 consumption and the redox potential of these cells. It was also found that the redox potential of sea water, as diluted by hypertonic NaCl, $CaCl_2$, $MgCl_2$, butyric acid or sucrose, became more negative than that of sea water alone. These facts were used as a basis for the hypothesis that a proper redox potential or ratio of oxidants to reductants of the respiratory enzymes was necessary for producing fertilization of the egg. The hypothesis as presented states that the redox potential of the external solution or sperm as compared with that of the exterior of the egg itself is an important factor in producing fertilization of the egg.

From the results with KCN in sea water which produced fertilization membranes but not cleavage, it was concluded that the formation of the fertilization membrane is not associated with oxidations, and appears rather to be due to change in the physical aggregation of some proteins at the surface of the egg or to a denaturation process occurring as the redox potentials is changing.

Dr. C. L. Yntema. No abstract submitted.

July 30

The action of napthoquinone antimalarials on respiratory systems. Christian B. Anfisen and Eric G. Ball.

In confirmation of the findings of Wendel (unpublished reports) a series of 2-hydroxy-3-alkyl-naphthoquinones have been found to exert a powerful inhibitory effect on the respiratory metabolism of the malarial parasite. The most powerful tested to date is the compound 2-hydroxy-3(2-methyl-octyl)-naphthoquinone-1,4 (M–285) which, at a level of 1 mg./liter inhibits *Plasmodium knowlesi* respiration 60 per cent. In experiments to localize the site of action of M–285 in the main respiratory chain of enzymes it was found that p-phenylene-diamine oxidation, requiring only the cytochrome system, was not inhibited by the drug, while the oxidation of succinate to fumarate by succinic oxidase prepared from beef heart was completely inhibited at about I mg./liter. The drug, therefore, appears to inhibit at an oxidation-reduction potention level below that of cytochrome C. Succinic dehydrogenase activity, as measured by the Thunberg methylene blue technique, was only very slightly diminished even at high drug concentrations. Similarly, the respiration of both fertilized and unfertilized eggs of *Arbacia punctulata*, neither presumably containing succinic dehydrogenase, was inhibited strongly at levels as low as 0.1 mg./liter (2×10^{-7} M.).This enzyme, therefore, does not seem to be the inhibited system. A number of flavoproteins including d-amino acid oxidase and xanthine oxidase, as well as several systems involving the mediation of the pyridine nucleotides, showed no decrease in activity in the presence of the drug.

It appears possible that the naphthoquinones under study are inhibiting a hitherto undetected enzyme or enzyme group in the main chain of oxidative metabolism having an E_o below that of cytochrome C and above that of the flavoproteins.

Chemical sense and taste in the Sea Robin, Prionotus. Ernst Scharrer.

The differentiation of taste and chemical sense is partly based on the concept that chemical sensitivity can evoke only negative or defensive reactions; positive reactions to food are mediated by the sense of taste (Kappers, Huber, Crosby, 1936, p. 347). Observations in the sea robin, Prionotus, do not support this conclusion. Prionotus possesses three free fin rays. Their epithelium is innervated by spinal nerves; taste buds are absent. The afferent fibers end in accessory lobes on the dorsal surface of the cephalic end of the spinal cord. Secondary fibers from these lobes which represent the greatly enlarged dorsal horns, ascend cephalad to the funicular nucleus from which fibers pass ventromedially, crossing in the ventral commissure, and ending in the contralateral ventral horn. When the free fin rays of blinded and sufficiently hungry sea robins are stimulated with extracts of clams or crabs the animals react positively by turning to and snapping in the direction from where the juice comes. Positive reactions to chemical stimuli are mediated in this case by spinal nerves and in the absence of taste buds. The differentiation between chemical sense and taste can, therefore, be based only on the innervation and the presence or absence of taste buds. The reaction of the animal cannot be used as a criterion.

Studies of the respiration of the imaginal discs of Drosophila using the Cartesian diver ultramicrorespirometer. CLAUDE A. VILLEE.

A determination of the effects of a mutant gene on the metabolic activity of a particular group of cells provides a basic approach to the analysis of gene action in development. In most animals it is impossible to locate exactly the cells which will give rise to a particular structure, but in Drosophila each organ develops from a discrete group of cells, an imaginal disc, which can be dissected out of the larva. The rate of respiration of wing and leg discs from wild, "miniature" wing, and "vestigial" wing stocks were determined by the Cartesian diver ultramicrorespirometer and their weights measured by the quartz fiber balance of Lowry. The legs of the adults of all three stocks are normal, the wings of adult "miniature" flies are about two-thirds normal size but of normal shape, and the wings of adult "vestigial" flies are small misshapen stumps, less than one-quarter the size of the normal wing. At each of several stages studied, before, at and after pupation, the Q_{O_2} of wild type discs and the leg discs of all stocks used varied only slightly from 20 cu. mm. O_2 per hour per milligram of tissue. The Q_{O_2} of "miniature" wing discs was 18 and of "vestigial" wing discs 9 cu. mm. O_2 per hour per milligram of tissue. The weights of "vestigial," "miniature" and wild type wing discs are the same at corresponding developmental stages in the larvae and early (1–2 hour) pupae. The discs contain considerable reserves of substrate and will respire in the divers twelve hours or more. The mutant genes "vestigial" and "miniature" produce their effects by altering the rate of some chemical reaction in the wing disc of the larva which is reflected by a lowered rate of oxygen consumption. These results do not mean that the "miniature" and "vestigial" genes affect the same chemical reaction in development to a different extent but rather that in affecting different processes they each lower the overall metabolic rate of the disc. The metabolism of the leg discs, and probably of the other discs as well, is not changed, although the cells contain the mutant gene. The "vestigial" and "miniature" genes therefore produce their physiological as well as their morphological effects only in certain cells of the body, presumably due to the interaction of the gene or gene products with specific components of the cytoplasm of those cells.

AUGUST 6

The specificity of chlorine esterase. PHILIP B. ARMSTRONG.

The relative rates of hydrolysis of choline esters acting at the nerve terminations in the sphincter pupillae of the turtle could be inferred by determining the relative potentiations by eserine of threshold concentrations for pupillary constriction of the choline esters. A comparison of the potentiations *in vivo* with the relative hydrolysis rates of the choline esters by the purified specific choline esterase *in vitro* indicates that the enzyme as it functions *in vivo* is as specific if not more so than *in vitro*. The choline ester substrate concentrations *in vivo* at which eserine was effective were much lower than those for effective substrate hydrolysis *in vitro*.

DR. T. H. BULLOCK. No abstract submitted.

The endocrine role of the corpora allata in insects. BERTA SCHARRER.

In *Leucophaea maderae* (Orthoptera) extirpation of the corpora allata at nymphal stages earlier than the last causes an abbreviation of development (suppression of molts) which results in animals with adult-like characters ("adultoids"). In operated seventh instars the following nymphal molt is suppressed, and the animals emerge as adultoids, resembling normal adults except for their smaller size and comparatively shorter wings. Allatectomized sixth or fifth instars result in "pre-dultoid" stages which show less adultoid differentiation and require an additional molt before becoming adultoids. In the adult insect the corpora allata are necessary for the development of the eggs. In females allatectomized shortly after the beginning of a reproductive cycle the eggs do not develop appreciably beyond the stage typical of the ovary at the time of operation. The accessory sex glands in these operated females show little or no sign of secretion in contrast to normal control glands. Reimplantation of the corpora allata into allatectomized females causes the eggs and the nymphs hatching from them to develop as normally as those of unoperated animals. In a series of experiments in which the time of allatectomy is varied it can be demonstrated that the corpora allata are necessary throughout the period of growth and yolk deposition which constitutes about the first third of the total period required for the development of the eggs. The corpora allata are apparently not essential for the reproductive activity of male Leucophaea. Allatectomized males when mated with normal virgin females are capable of fertilizing the eggs.

Contrasts between visible and dominant lethal mutation rates in x-rayed Habrobracon eggs. ANNA R. WHITING AND H. C. GEORGE.

Senior author has previously reported that eggs x-rayed in late metaphase I have lethal dose about 2,000 r and one-hit dose-hatchability curve. Death appears to be due to terminal deletions. Eggs x-rayed in prophase I have lethal dose about 45,000 r and complex dose-hatchability curve. Death appears to be due to several factors, including translocations and inversions. Majority of lethal effects in both stages are dominant. Recently, two groups of females were treated with doses giving about 90 per cent mortality, one with 1,120 r for metaphase I and the other with 28,000 r for prophase I. They were then crossed with untreated males and their daughters were tested for heterozygosis for visible mutations. $\frac{\text{F}_1 \text{ ♀♀ heterozygous}}{\text{F}_1 \text{ ♀♀ tested}}$ was 2.11 per cent for eggs treated in metaphase I, 12.69 per cent for eggs treated in prophase I. By χ^2 test there is less than one chance in one hundred that these stages belong to same class in respect to visible mutation rate although they have same dominant lethality rate. This strengthens theory of terminal deletions (which would not produce visibles) as most common response to x-rays of metaphase I. Visibles produced in metaphase I are probably genic and their low percentage is what would be expected at low doses tolerated by this stage. Most visibles from treated prophase I are probably also genic although a few may be due to position effects of translocations or inversions. Their high percentage is possible because of high doses tolerated.

AUGUST 13

A new factor from the adrenal influencing fat deposition in the liver. KATHERINE A. BROWNELL.

Starvation in the normal mouse leads to a large deposition of fat in the liver. This fails to occur after adrenalectomy. With these facts as a basis we have developed a test for a fat factor in various fractions prepared from ox adrenals.

The method is briefly as follows: Adrenalectomized mice are fed for 24 hours then fasted for 24 hours. During this 48-hour period they are injected every 6 hours with 0.2 cc. of the preparation to be tested. Two to 3 hours after the final injection the livers are removed and the total lipid determined gravimetrically.

Over 30 fractions from the adrenal gland including crystalline compounds have been tested by this method. The table shows results on adrenalectomized untreated animals; two fractions, a whole extract from which these fractions were taken and three crystalline compounds already proven to have glyconeogenic potency. Both fractions are crude, being specific in only one respect—namely, that the carbohydrate factor fraction has no electrolyte potency and the sodium factor fraction no glyconeogenic potency. The only fraction that gave a highly significant response was that containing the carbohydrate factor. The low response given by whole extract, we attribute to inhibiting substances, three of which have been tested.

Since the liver fat response was given almost exclusively by the carbohydrate factor fraction, some of the crystalline compounds having glyconeogenic properties were tried to determine whether or not they were responsible. The table shows that the only one used which gave a significant response was dehydrocorticosterone; a 25 per cent increase over the control level and in order to obtain this response two and one half times as much pure substance (0.96 mgm.) was used as that estimated to be present in our carbohydrate factor fraction (0.35 mgm.). The other two compounds, corticosterone and 17-hydroxy-11-dehydrocorticosterone, gave liver fat responses only on the borderline of significance and to obtain even these small responses two to two and one half times as much material was used as that estimated to be present in the carbohydrate factor fraction. The fourth known glyconeogenic compound, hydroxycorticosterone, we were unable to test on account of lack of material.

There remain two possibilties: (1) that hydroxycorticosterone is the fat factor. If so, the effect on fat metabolism is a new property. (2) There is in the carbohydrate factor fraction a new factor regulating fat deposition in the liver.

Effect of adrenal fractions on deposition of fat in the liver

Treatment	No. of animals	Total lipid per cent	Increase per cent
Adect. untreated	29	6.31	—
Carbo. factor fraction *	15	8.42	33
No factor fraction *	7	6.74	9
Whole extract *	7	7.13	13
Dehydrocorticosterone †	8	7.87	25
Corticosterone †	8	7.11	13
17-hydroxy-11-dehydrocorticosterone †	7	6.87	9

* The extracts represent 300 gm. of tissue per cc.

† The solutions of crystals represent 0.6 mgm. solid per cc.

Hyperactivity of the adrenal cortex. Frank A. Hartman.

At rest or under conditions of minimal activity there is a basal secretion of adrenal cortical hormones. In response to various stresses such as exercise, exposure to cold, trauma, anoxia, and poisons, there is an increase in output of the hormones which subsides after the stimulus disappears. After removal of a large proportion of both adrenals by enucleation, in the mouse, a considerable rise in the basal secretion occurs. This higher level of secretion is maintained for months. The following table illustrates these changes. Fat and glycogen (as sugar) in the liver were determined after 24 hours' starvation.

Values indicating changes in hormone production after enucleation of both adrenals

	Total lipid per cent	Glycogen per cent
Normal	8.5	0.12
Adrenalectomized	6.3	0.04
Enucleated 2 days	6.6	—
Enucleated 7 days	11.8	0.24
Enucleated 15 days	10.0	—
Enucleated 29 days	—	0.58
Enucleated 99 days	10.0	—

The wide difference in time at which the peaks for the production of the fat factor and carbohydrate factor occur, is evidence that the two factors are not identical.

By enucleation we removed an average of 75 per cent of the adrenal tissue. Less than 25 per cent of the active tissue remained since the circulation was disturbed and this 25 included the capsule. Thirteen days after enucleation the adrenals averaged 0.69 per cent of the body weight which is one-half the normal weight. Removal of cortical tissue probably reduces the inhibitory effect on the adrenotrophic hormone production by the pituitary so that after a lag of three or four days there is sufficient recovery of the remaining cortices to respond to the increased output of adrenotrophic hormone. However, the new level of cortical hormone production does not return the adrenotrophic output to the old level. Thus a higher basal level is established. The performance of a relatively small number of cortical cells indicates a large factor of safety. This capacity of cortical cells for sustained activity in disease where a large proportion of cortical tissue is destroyed is important in prolonging life.

There is now evidence for three mother hormones secreted by the adrenal cortex; the fat factor, the carbohydrate factor, and the sodium factor.

Studies on the mechanism of alloxan action. Arnold Lazarow and Stanley Levey.

A number of compounds related to alloxan were synthesized and tested for their diabetogenic effect. These compounds were injected intraperitoneally into rats in high doses and the blood sugar was determined at 0, 1, 3, 8, 24, 48, and 72 hours after injection. Alloxan, N-methyl alloxan, and alloxantin which dissociates into alloxan all produced diabetes. N-N-dimethyl alloxan was toxic and, therefore, could not be injected in doses equivalent to that required for the production of diabetes with alloxan. Since alloxan is a ureid of mesoxalic acid, some derivatives were prepared in which the urea or mesoxalic acid portions of the molecule were intact. None of these (mesoxalamide, mesoxalic acid, dimethyl mesoxylate, or diacetyl urea) produced diabetes in the doses used. Freshly prepared dialuric acid, alloxanic acid, and barbituric acid did not produce diabetes; whereas, dialuric acid which was allowed to stand overnight was diabetogenic. (This is interpreted as oxidation of dialuric acid to alloxan by molecular oxygen.) Slight alterations in the structure of alloxan abolish its diabetogenic effect.

It has been reported by other investigators that alloxan combines with sulfhydryl groups of proteins and that on injection it produces a rapid drop in the blood and tissue glutathione. Since one of us has shown that injection of glutathione or cysteine immediately preceeding a diabetogenic dose of alloxan completely protected the animals from diabetes; and since others have shown that pancreas contains less glutathione than do other tissues; it was suggested that variations in tissue glutathione may determine the selectivity of alloxan. Studies are now being carried out to determine the glutathione content of the beta cells of the pancreas which are selectively destroyed by alloxan.

Biological specificity and the synthesis of native proteins. Dorothy Wrinch.

A common starting point for the discussion of biological specificity today is the assumption that biological function is an outward and visible sign of atomic pattern. Furthermore indications from many fields reinforce the old assumption that the native protein is the dominant structure type in all living systems. A vast number of physiological problems turn upon questions of atomic pattern, particularly such matters as (1) local stereochemical features and (2) the presence of internal OH...O, NH...O and NH...N bridges and of linkages dependent upon the presence of a foreign ion.

Of all these problems, the most fundamental is the synthesis of native proteins. We must presume that the power of native proteins to produce replicas of themselves depends in some basic way upon their structure, and that it is intimately related to the presence on native protein surfaces of 'active patches' to use Warburg's term, each of which functioning as a template or mold permits the laying down on itself of a complementary constellation.

It is useful to notice that the associations of simple molecules within crystals offer many examples of such complementary constellations, e.g., (1) hexamethylene tetramine, with pairs which are not identical associated about tetrahedrally related planes with a common three-fold axis and (2) the phosphotungstic acid 29-hydrate with identical (i.e., self complementary) constellations associated about such planes with a common three-fold axis and, in addition, self-complementary constellations associated about cube planes with a common two-fold axis.

Visualizing the formation of new 'active patches' on the surfaces of an already existing species of native protein molecules, we see that such new constellations comprise the material required for the formation of a new and identical molecule if (1) the species carries complementary patches (which may be but need not be individually self-complementary) and (2) the molecule is wholly made up of such patches, i.e., is a *surface structure*.

In order to have a mechanism whereby these isolated constellations on several different molecules may be integrated so as to interlock in the same spatial pattern as in the original molecule, something has to be postulated as to the capacity of the original molecules to form a crystal. Thus for example, let us visualize a body-centered cubic lattice with molecules placed at the 8 body-centers and the 6 cube corners nearest the origin, with the molecule at the origin missing. With the complementary constellations in position on each of the $8 + 6$ faces of the molecules turned to the origin, we have a situation in which interlocking, possibly in a number of distinct steps, could take place, the resulting molecule being a replica of the original molecule. This is but one example of a number of such possibilities, with the original molecules characterized by antipodal pairs of complementary patches. All, however, have in common the dependence upon the capacity of the original molecules to crystallize, an outstanding characteristic and most remarkable property of unnumbered native proteins. Similarly, all theories as to the formation of new native protein molecules by autocatalysis must, it would seem, have in common the picture of such molecules as surface structures, i.e., atomic fabric cages.

AUGUST 20

Naturally occurring polyploidy in the development of Allium cepa L. Dr. C. A. BERGER.

One of the factors in the developmental pattern of *Allium cepa* is the formation of some tetraploid cells and their division as tetraploids. These cells are found throughout the cortex of the cotyledon and of the intermediate region between root and shoot. They are found in seedlings between 20 and 40 mm. in length. They are never found in the root. During prophase of mitosis in tetraploid cells the chromosomes are closely paired and relationally coiled. The two members of each pair are united at a single undivided SA-region. These cytological details show that the chromosomes have not separated since the time of their formation. Since the pairing and relational coiling is present from earliest prophase the double chromosome reduplication must have taken place during the resting stage immediately preceding the 4n division. At metaphase the tetrachromosomes undergo two successive divisions of the SA-region and anaphase is normal. Since no tetraploid division figures were found with unpaired chromosomes it was concluded that only one division of these tetraploid cells occurs.

Chick embryology at the medical schools of Ancient Greece. TAGE U. H. ELLINGER.

Of the seventy titles comprising the Hippocratic Corpus, the most significant work, from a biological standpoint, is the lecture on embryology represented by the two texts *On Semen* and *On the Development of the Child.* It deals with human embryology from the formation of the semen to the birth of the child. The author is unknown, but he was not Hippocrates nor one of his followers. His work reflects the teachings of the medical school at Cnidos and of that of Empedocles whose influence is evident in doctrine as well as in scientific method and in the choice of vocabulary. This pre-Aristotelian author, who wrote in the last quarter of the fifth century B.C., at the time of Socrates, was indeed a very great scientist and a great teacher as well.

To the modern reader perhaps the most amazing revelation is the use made of observations on chick embryology in explaining to the students the development of the human embryo. The following quotations are in the author's translation.

In chapter 13, the Greek physician after describing a "semen which had stayed six days in the womb and which fell out," adds "A little later I will describe another test in addition to this one, that will enable anyone who seeks knowledge to see this for himself, as well as a proof that my whole discourse is correct, as far as that is possible for a mortal discussing such a matter."

He returns to this topic in chapter 29: "Now I shall recount the crucial test, that I promised a little while ago to make known, which is as clear as possible to a human intelligence and makes plain to anyone who wants to be informed about it, that the semen is in a membrane and that the navel is in the middle of it, and that it first draws air in and expels it outward," (according to the Empedocles' pneuma theory of differentiation) "and that there are membranes from the navel. You will also find the further growth of the child, as I have described it, to be from beginning to end, such as it is in my account, if you will apply the method of inquiry that I am about to describe. Take twenty eggs or more and give them to hatch to two hens or more; then on every day from the second to the last, that of hatching, remove an egg, break it and examine it. You will find that everything in it conforms with my statements, in so far as one can compare the growth of a bird with that of man. That there are membranes extending from the navel, and all my other statements about the child, you will find illustrated from beginning to end in the hen's egg; and he who has not yet made these observations will be surprised that there is a navel in a hen's egg. Such are the facts, and such is my account of them."

Again in chapter 30, the Greek author advances chick observations to illustrate and explain conditions in man. He states: "Now in proof of my theory, that it is the lack of nourishment that causes the child to come forth, provided it suffers no violence, I offer the following evidence. The bird develops from the yolk of the egg in the following way. Under the brooding mother the egg is heated and the content of matter inside receives the impulse to development from the mother. When the content of the egg is heated, it forms air and attracts other cold air from the atmosphere through the egg; for the egg is porous enough to admit the attracted air in sufficient quantity to the matter inside. The bird grows in the egg and is differentiated in the same or in a similar way to the child, as I have already said above. It develops from the yolk, but it receives its nourishment and material for growth from the white that is in the egg. This was at once apparent to all those who have given attention to it. Whenever nourishment from the egg is insufficient for the chick, then, not having sufficient nourishment to live on, it moves violently in the egg seeking more nourishment, and the membranes about it burst. When the mother notices that the chick has moved violently, she pecks and removes the shell. And this happens in twenty days. And it is evident that this is so, for, when the mother pecks the shell of the egg, there remains in it no liquid worth mentioning, since it has been expended on the chick."

Reproductive economy in closecrossed species with haploid males. P. W. WHITING AND RUDOLPH G. SCHMEIDER.

According to the multiple-allele theory of sex determination, proved true for the wasp Habrobracon, every mating must involve either three or two sex alleles. The three-allele matings produce only females (sex heterozygotes) and normal (haploid) males (azygotes); but the two-allele matings produce also sex-homozygotes which either develop into sterile (diploid) males or are inviable. Outcrossing reduces the chance for two-allele crosses with their attendant reproductive wastage. The Habrobracon theory has been tentatively applied to the six or seven invertebrate groups characterized by male haploidy. Since many species, however, reproduce with much inbreeding, this theory would imply loss approximating half of the fertilized eggs. It has now been shown that in the wasp Melittobia over 90 per cent of the eggs from closecrosses, including selfcrosses (mother × haploid son), may develop into females. If Melittobia females are sex-heterozygotes, some method must therefore have been evolved other than multiple alleles for avoiding production of sex-homozygotes equal in number to the females. Although the method of sex determination in Melittobia is not yet understood, it has now for the first time been shown that reproductive economy is high in a closecrossed species with haploid males.

A comparative study of the lipids in some marine annelides. CHARLES G. WILBER.

Studies on the metabolism of lipids have been in the past confined to observations made on vertebrates. Very few studies have been made on the lipids in the invertebrates; consequently a detailed investigation seems justified.

The following marine annelides were studied: *Nereis pelagica, Amphitrite ornata, Arenicola marina, Phascolosoma gouldii, Lepidonotus squamatus, Glycera americana,* and *Chaetopterus variopedatus.*

Whole worms or individual tissues were prepared by grinding or in the Waring-blendor. Lipids were extracted with boiling alcohol. Phospholipids were precipitated with acetone and magnesium chloride and estimated by oxidation-titration method of Bloor. Fatty acids were estimated by oxidation-titration and cholesterol colorimetrically using the acetic anhydride-sulfuric acid reagent. The ratios, cholesterol/fatty acid (lipocytic index) and cholesterol/phospholipid, were calculated.

It was found that the absolute values of the various lipids in the same species and in different species were not always the same. On the other hand, the lipocytic index and the relation, cholesterol/phospholipid, were constant for a given species and tissue. If the lipocytic index of each worm were plotted against the phospholipid of the same worm the points representing the various species fell along a straight line; a similar straight line was obtained when the cholesterol was plotted against phospholipid.

There is, therefore, an apparent relationship between cholesterol and phospholipid and between phospholipid and lipocytic index in marine annelides. Tissues with a high lipocytic index or high cholesterol content have a high phospholipid content. These results indicate that in the marine annelides, just as Bloor found in the vertebrates, since cholesterol is associated with and in constant relation to phospholipids, it is probably a normal protoplasmic constituent. These results confirm in part the results of analyses made on vertebrate tissues and agree with the contention of Mayer and Schaeffer that the lipocytic index is characteristic of the organ of an animal in a given species.

GENERAL SCIENTIFIC MEETINGS

August 23

*Vascular reactions to ergonovine maleate * as seen directly with the microscope in the living mammal.*[1] Richard G. Abell.

Erognovine was injected intravenously in amounts varying from 0.005 mgm. to 0.2 mgm., and its effect upon the arterioles, capillaries and venules observed directly with the microscope in transparent 'moat' chambers (Abell and Clark, '32) in rabbits' ears. The clinical intravenous dose of ergonovine is 0.1 mgm. The equivalent dose in the rabbit is approximately 0.005 mgm. Injections of 0.005 mgm. caused constriction of arterioles to approximately 0.7 to 0.9 of their control diameters, and a slight reduction in velocity of flow. The arterioles returned to their control diameters and the flow to its control rate within 3 to 5 minutes. Daily injections of 0.005 mgm. made for a period of 2 weeks caused similar results. One hundredth mgm. (twice the clinical dose) caused constriction of the arterioles to approximately 0.6 to 0.8 of their control diameters, and a slightly greater reduction in rate of flow than 0.005 mgm. One tenth mgm. (20 times the clinical dose) caused arterioles 15 to 30 microns in diameter to constrict to the point of obliterating their lumens and stopping the blood flow for approximately 30 seconds to one minute. The vessels relaxed to their control diameters within approximately 12 minutes. Two tenths mgm. (40 times the clinical dose) caused more vigorous and prolonged arteriolar constriction, which lasted for from 1 to 1½ minutes, and stopped all of the blood flow within the chamber. The venules constricted to approximately 0.6 to 0.7 of their control diameters. The arterioles returned to their control diameters in approximately 15 to 20 minutes. Four injections of 0.2 mgm. at 15 minute intervals made the small arterioles (15 to 30 microns in diameter) unresponsive to further injections, but not the larger arterioles (80 to 90 microns). Intravenous injections of 0.025 mgm. of epinephrine while the small arterioles were still unresponsive to ergonovine, caused them to constrict to the point of obliterating their lumens, which is the typical response to this amount of epinephrine.

None of the above injections caused any sign of injury to the blood vessels, or any abnormalities in appearance and distribution of the red blood cells, the white blood cells, or the platelets. Thus it is clear that ergonovine maleate, which is used widely to prevent post partum hemorrhage and to give symptomatic relief of migraine headache, does not cause any observable injury to the blood vessels and associated structures even when given in amounts of 40 times the clinical dose.

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* "Ergotrate" (Ergonovine Maleate, U.S.P., Lilly).

[1] This work was aided by a grant made by Eli Lilly and Company to the Department of Anatomy of the University of Pennsylvania Medical School.

The effect of halogenated alkyl amines on the respiration of Arbacia eggs and sperm. E. S. Guzman Barron, E. G. Mendes and H. T. Narahara.

Halogenated alkyl amines at 0.001 M concentration produce an inhibition of the respiration of animal tissues, and complete inhibition of pyruvate and choline oxidation (Barron et al.[1]). In smaller concentrations the early cleavage of the fertilized sea urchin egg is inhibited or retarded (Cannan et al.[1]). There is also inhibition of mitosis in the corneal epithelium of mammals (Friedenwald and Scholz[1]) and a high incidence of sex-linked lethals as well as a significant number of translocations and inversions in the chromosomes of *Drosophila melanogaster* (Auerbach et al.[1]).

Dichloroethylmethylamine HCl, and trichloroethylamine HCl at a concentration of 0.001 M. and dissolved in sea water, produced a definite increase in the respiration of sea urchin sperm (from 170 to 50 per cent). The increase of respiration could be noticed even with 1×10^{-5} M. The respiration of sea urchin eggs, fertilized or unfertilized, was slightly inhibited by this concentration of alkyl amine (14 to 17 per cent). Higher concentrations produced inhibition of respiration probably due to a decrease in pH as a result of the hydrolysis of these compounds. When the alkyl amines were previously neutralized and the sperm and eggs suspended in 0.05 M citrate buffer, pH 6.8, the effect of the alkyl amines was erratic. It is quite possible that penetration of the alkyl amines into the cell occurs only in an acid milieu.

The experiments of Cannan et al.[1] on retardation of the rate of cleavage of fertilized Arbacia eggs were confirmed. Eggs treated with 0.001 M dichloroethylmethylamine HCL (dissolved in sea water) for 15 minutes prior to insemination, and fertilized eggs treated at the time of the first cleavage showed a definite retardation in the rate of cleavage. Furthermore none of the treated eggs reached the pluteus stage.

The effect of uranyl nitrate on the respiration of Arbacia sperm. D. Benedict and E. S. G. Barron.

Uranium, like other heavy metals, is quite toxic and it has been extensively used for the production and study of experimental nephritis. Uranyl nitrate in concentrations varying from 10^{-2} to 5×10^{-5} M. inhibited the respiration of Arbacia sperm. The inhibition was complete at 5×10^{-4} M. (92 per cent inhibition). 10^{-4} M. $UO_2(NO_3)_2$ produced partial inhibition (from 53 to 15 per cent), 5×10^{-5} M. inhibited 15 per cent, and 10^{-5} M. had no effect at all. This inhibition must be due to combination of respiratory enzymes with uranium, a combination which can be reversed completely on addition of a citrate at a ratio of U : citrate of 1 : 2. Addition of phosphate at a ratio of 1 :100 brought only partial release (25 per cent). The experiments were performed in acetate-sea water buffer at pH 6.4 to avoid precipitation of the uranyl salt. Dry weights of sperm were obtained after centrifugation of the sperm at 16,000 g. There was in the control experiments a rise in the pH value of about 0.6 units at the end of one hour, probably due to the formation of NH_3.

Some properties of purified squid visual pigment. Alfred F. Bliss.

The photostable red visual pigment of the squid (Bliss, 1943, *Jour. Gen. Physiol.*) was found to become reversibly light sensitive in the presence of formalin. A method was devised for the extraction of this pigment in a state of purity approximating that of the best preparations of vertebrate rhodopsin. The principal impurity of previous extracts, melanoprotein, was rendered insoluble by the following procedure. Retinas were rinsed in distilled water and kept frozen until use. They were then homogenizd with 0.2 M $Na_2\ HPO_4$ and centrifuged. The residue was washed with pH 4.5 buffer and distilled water. The visual pigment was extracted with 3 per cent digitonin at 6° C. for 2 minutes and centrifuged 5 minutes. The absorption spectrum of the extracted pigment did not differ significantly from that of rhodopsin. In its chemical properties it differed significantly from rhodopsin, since it was rapidly destroyed by digitonin even at 6° C. The primary breakdown product in cold acetone was, like that of rhodopsin (Bliss, 1946, *Biol. Bull.*), the acid tautomer of the lipid "Indicator Yellow." Because of the distinctive properties of the squid rhodopsin, a differentiating name, cephalopsin, is suggested.

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[1] All quoted from Gilman, A., and Philips, F., *Science* **103**: 409 (1946).

Studies on the viscosity and elasticity of striated muscle. MANFRED BRUST.

By the use of a spring vibrating against the resistance of frogs' (*Rana pipiens*) sartorius muscles—as described by Gasser and Hill (*Proc. Roy. Soc. B.* **96**: 398, 1924)—the effects of urea and iodoacetic acid (IAA) on the viscosity and elasticity of these muscles were studied. All initial slack was removed from the system by stretching the muscles 17.5 per cent beyond their resting length and putting them under 3.5 gm. tension.

Thirty minutes immersion in solutions of 2.5 M urea in Ringer's shortens the muscles on the average by 26.1 per cent. When extended to their original length they still exert the tension originally exerted at that length. They will not return to their urea induced length when released from stretch. Their viscosity is reduced on the average of 53.6 ± 13.0 per cent of that in the untreated muscles, while the elasticity is similarly reduced to 60.4 ± 18.6 per cent.

Sixty minutes immersion of Ringer's equilibrated muscles in 1-80 000 IAA (6.72×10^{-5} M) in Ringer's sometimes causes a rise in viscosity and elasticity even without activity by the poisoned muscles. Summer frogs show this response less often than winter frogs. Measurements made during 30 second rest periods between 5 second isometric tetani show a short initial decrease followed by a gradual increase in both viscosity and elasticity. Average maximum rigor values of 181 per cent and 258 per cent respectively of the untreated muscle values are attained.

The urea results would agree with the findings by other authors that this agent disrupts myosin and other protein molecules thus transforming them into disconnected less asymmetric entities. Collagen is not believed to be markedly affected since muscle shape is maintained while tension remains the same as before treatment at the same lengths. The IAA results would agree with the progressively diminishing solubility and increase in hardness of actomyosin in gradually decreasing concentrations of adenosine triphosphate reported by the Szent-Györgyi group (*Acta Physiol. Scand.* **9**: *Suppl.* xxv, 1945).

Arterial anastomoses. ELIOT R. CLARK AND ELEANOR LINTON CLARK.

This study represents an attempt to discover factors responsible for the presence or absence of arterial anastomoses, which vary so greatly in different organs.

The governing factor appears to be the histo-mechanical principle established by R. Thoma in 1892, corroborated by E. R. Clark in 1918 in studies on living vessels in the tadpole's tail, that the size of the lumen of an artery is regulated by the amount of blood flow. In the absence of flow, the lumen is reduced to zero and the artery obliterated. In order, then, for arterial anastomoses to survive, conditions must be such as to provide a flow of blood through the terminal connecting portion.

In most cases this requires the presence of factors which force the blood to flow part of the time in one and part of the time in the reverse direction. Such factors are present in the peripheral parts of the body in the form of varying outside pressures that are exerted irregularly upon large supplying arteries or small distributing arterioles.

A study, with the aid of artificial chambers, of the living circulation in the rabbit's ear, where anastomeses are abundant, reveals frequent reversals of flow in connecting portions of anastomoses, but controlled by an unsuspected factor, namely, the irregular contraction of the arteries or arterioles themselves, described in an earlier paper.

In types of artificial chambers, installed in rabbits' ears, which are invaded by new tissue, there are often arterioles that, for weeks, are unprovided with nerves and hence contract little, if at all. In many such chambers no arterial anastomoses survive. However, in this type of chamber, occasionally arterioles receive a nerve supply, and in such cases arterial anastomoses may survive. In every case in which such anastomoses have persisted in newly-formed tissue, there have been frequent reversals of flow in the connecting portion.

The effects of the ultra-violet radiations on Styela eggs. A. M. DALCQ.

The M.D.L. installation for microphotography with U.V. rays (2537 Å) may be used for irradiating part of the Ascidian egg or certain of the various blastomeres up to the VIII-cell stage. The method was worked out with the aid of Dr. G. I. Lavin. The egg is placed in a drop of sea water near the edge of a thin quartz coverslip, which is itself put on the transverse arm of the mechanical stage. The coverslip is adjusted under the microscope in such a way that the part of the egg to be irradiated protrudes over the edge of the metallic stage arm which acts as a protection screen for the rest of the egg. Attention should be paid to two sources of error: (1) the effect of hypertony due to evaporation of the drop and (2) to the reflexion of the rays by the objective lens of the microscope, which is easily eliminated by interposing some black paper during the irradiation. In exploring a considerable range of exposure no favorable effect of the irradiation could be found. If feeble, it produces a delayed disorganization of the embryonic layers. If stronger, it stops the cleavage with rapidity varying according to the dosage. In order to obtain stopping of the next cleavage, exposures of at least 10 minutes are necessary. When a division is suppressed, the cell may manifest a delayed attempt at cleavage, but this is always abortive. After exposure of the unsegmented eggs, deviations of the first cleavage plane may be observed. Observations of the movements of yolk and yellow pigment and the elongation of the cell-body indicate that the effect of the radiation is not primarily on the nuclear activity. That the influence of the rays is exerted on the surface protoplasm is shown by the transitory appearance of alterations of the surface film (small protuberances, "blisters") in coincidence with attempts at cleavage.

By means of this method, the division of one or more blastomeres of the II, IV, and VIII cell stages has been inhibited. The non-irradiated cells exhibit normal development with respect to mitotic rhythm and arrangement. Their capacity for differentiation, which seems rather poor when large blastomeres remain undivided in the germ, must still be studied in sections.

A correlation between gill surface and activity in marine fishes. I. E. Gray.

The units of respiration in the gills of fishes are the numerous microscopic secondary lamellae which appear as thin, leaf-like plates set at right angles to the main axes of the primary lamellae. Within each plate lies a capillary network through which the interchange of gases takes place. Among fishes there are species differences, not only in the number of gills, but also in the number and length of the gill filaments (primary lamellae) and in the number of respiratory units (secondary lamellae). By determining the number of respiratory units per gram of body weight it is possible to obtain an estimate of the relative respiratory ability of different fishes.

There is a marked contrast in the number of respiratory units per gram of body weight between the active, surface, migratory fishes (mackerel, 2550; butterfish, 1725; menhaden, 1685) and the sluggish bottom fishes (flounder, 265; toadfish, 135; goosefish, 50). The number of respiratory units of fishes of medium activity fall between these two extremes (scup, 1325; sea trout, 1250; sea bass, 1110; eel, 900; sea robin, 800; puffer, 505; tautog, 440). A four hundred gram mackerel has a total of nearly three-fourths million respiratory units while a toadfish of the same weight has only fifty thousand. The number of respiratory units is also directly correlated with the amounts of sugar and hemoglobin in the blood.

The distribution of lipid between the light and heavy halves of the Arbacia egg. F. R. Hunter and A. K. Parpart.

Unfertilized *Arbacia* eggs were centrifuged for 10–20 minutes in an air turbine at approximately 16,000 × G. in a medium of graded density obtained by mixing sea water and 0.95 molal sucrose. The light and heavy halves which resulted were collected, packed in an air turbine, frozen, dried in a vacuum desiccator and weighed. This dried material was then extracted with ether, dried and again weighed. The loss in weight was taken as a measure of the amount of free fats and sterols. This material was then extracted with alcohol-ether and again dried and weighed. This was considered to give a value for the bound lipid. In order to relate the amount of lipid to the number of halves, counts were made on suspensions of halves prior to drying. The following values expressed as mgs. of lipid per million halves were obtained: heavy halves—6.6 (ether fraction), 12.2 (alcohol-ether fraction); light halves—2.2 (ether fraction), 9.6 (alcohol-ether fraction). Thus, 75.0 per cent of the free fats and sterols, 56.0 of the bound lipids and 61.6 per cent of the total lipids are in the heavy halves. The sum of the total lipids in the two halves is equal to 30.6 mgs. per 10^6 cells which compares favorably with the value 34.1 mgs. per 10^6 cells calculated from the data given by Parpart (*Biol. Bull.*, 81: 296, 1941) for unfertilized, whole eggs. Similarly a comparison can be made between the sum of the bound lipids of the two halves and of the whole egg. Their values are 71.3 per cent and 77 per cent, respectively.

Evidence for enzymatic participation in the penetration of the human erythrocyte by glycerol. PAUL G. LEFEVRE.

Jacobs and his associates have reported that an amount of copper sufficient to cover only a very small fraction of the surface of the cells involved markedly inhibits hemolysis of human red cells in isotonic glycerol. This report concerns the extension of this finding to the effects of other substances which inhibit the same types of enzymes affected by traces of copper.

Following the pattern prescribed by Barron and Singer for identification of sulfhydryl activity, iodine, mercuric ion, the arsenical Mapharsen, and p-chloromercuribenzoate were shown to inhibit hemolysis by glycerol, buffered at pH 7.1. This inhibition failed in the presence of cysteine, glutathione, or thioglycolic acid; and could be reversed by later addition of these substances at 2–3 times the concentration of the inhibitor, except with Mapharsen. These relations indicate strongly that active $-$SH groups are involved in carrying glycerol into the cell. Though sensitive to the inhibitors mentioned, the hemolytic process was not affected by iodoacetate; this indicates that the sulfhydryl groups involved are of the difficultly available type, not inactivated by the alkylating agents.

Since phosphorylation is apparently essential in transfer of sugars and other substances across the membranes of the kidney tubule and the intestinal cell, it is proposed tentatively that the enzymatic step involved in the present studies is the phosphorylation of glycerol. Adenosine triphosphatase, capable of this step, is present in the erythrocyte, and shows the same pattern of sensitivity to inhibitors as found in the present instance, as well as similar relations of activity to pH. Further, more decisive tests of the proposed identity of the enzymatic factor are planned.

"Accommodation" and opening excitation in nerve and muscle. PAUL G. LEFEVRE.

In his mathematical analysis of electrical excitation in 1936, Hill pointed out that the accommodative process (recession of threshold under the influence of a stimulus) itself accounted for the phenomenon of excitation at the anode at the "break" of a constant current. There seems to have been no attempt to test this neglected implication of Hill's theory: that "accommodation" is an essential prerequisite for "opening excitation" at the anode. This report concerns the occurrence of opening excitation in tissues showing no accommodation.

Following Solandt's practise, frog sciatic nerves were treated with citrate until they no longer showed any accommodation: their threshold was independent of the rate of increase of the excitatory current (delivered with Solandt's condenser-charge arrangement). In such preparations, in spite of the absence of accommodation, there was no difficulty in eliciting an anodal response at the cessation of a steady current. The same result was readily obtained with exposed sciatic nerves of anesthetized rats treated with citrate.

Frog sartorii, or the pharyngeal retractors of *Thyone,* if stimulated in the nerve-free regions, or following neural degeneration, also showed no accommodation. But all attempts to demonstrate any response at "break" in these muscles failed; this is in accordance with the predictions of Hill's theory. Only in the case of citrated nerves was seen the troublesome occurrence of opening excitation in the absence of any accommodation.

A further analysis of this matter is planned, to determine whether the results may be explained on the basis of a postulated fundamental difference between accommodation at the cathode and that at the anode; the latter persisting in the absence of the calcium ion required by the former.

A photometric study of the kinetics of fibrin formation. JOSEPH LEIN.

The clotting of fibrinogen solutions by thrombin was studied by measuring the optical density and light scatter as the process occurred. The results can be analyzed kinetically only when purified preparations were used. If other plasma proteins are present the degree of light scatter also depends on their concentrations. This is believed due to a trapping effect of non-clottable proteins by the fibrin as it is formed. The light scatter studies were particularly useful in the kinetic analysis of the clotting process.

The reaction was considered from a polymerization viewpoint, the fibrin representing the polymer formed through the action of thrombin on the monomer fibrinogen. First order reaction kinetics were employed. The following assumptions were made. (1) With constant thrombin concentrations the rate of increase of the polymer size is proportional to the fibrinogen concentration ($dN/dT = K_1F$). (2) The rate of decrease of the fibrinogen is proportional to the fibrinogen concentration ($-dF/dT = K_2F$). (3) The light scatter under the conditions of the experiment is proportional to the increase in particle size once it reaches the critical size (N_0) which first scatters light. ($LS = K_3(N - N_0)$. From these formulas a relationship was derived that included light scatter (LS), the initial concentration of fibrinogen (F_0), time (T), and time (T_0) for the polymer to reach a size (N_0) that would scatter light. The formula may be expressed as:

$$\log \left(K_1K_3/K_2 \; F_0e^{-K_2T_0} - LS \right) = -0.434 \; K_2T + \log \; K_1K_3/K_2 \; F_0$$

The relationship was tested on a series of experiments in which the initial concentration of fibrinogen was altered, the thrombin being kept constant. The calculated values of the constants agreed with the experimental values within a 6 per cent average deviation. It thus appears that the course of the reaction may be considered to be molecular and that thrombin acts as a true catalyst, not forming part of the final fibrin product.

The effect of iodacetate on the changes in muscular latency induced by activity. A. SANDOW. No abstract submitted.

Formation of the nuclear membrane and other mitotic events in Chaos chaos Linn and Chaos neos (new species). A. A. SCHAEFFER.

The mitotic stages of the amebas mentioned are easily followed in the living animal. The principal stages are the following: 1. the nucleus about to divide swells up to about 6 times its former volume. 2. The chromatin grains (300 to 600 in number) gradually disappear, as if going into solution. Some of these grains coalesce before going into solution. 3. A new mass of small grains (about 2500) appear, before all the larger grains of the so-called resting nucleus have disappeared. These small grains arrange themselves first as a lens-shaped cloud, then as a plate of about 2 grains thickness. At this stage the plate of grains is occasionally seen to be indistinctly divided into at least 4, possibly as many as 8 or 12, smaller groups of equal size. 4. This plate of grains then separates into 2 plates which rapidly move apart. 5. Fibers analogous to, if not identical with, spindle fibers, appear between the plates, as the plates separate. Fibers also appear on the other face of the plates. All fibers are at first horizontal and parallel. 6. The plates separate and the inter-plate fibers lengthen until the plates are separated to about 2 or 3 times their diameter when, because of the streaming of the protoplasm, the plates are torn apart. During this time the nuclear membrane breaks into pieces which eventually completely disappear. 7. The separated plates, still granular, become bent like a concavo-convex lens, with polar fibers still attached. The granules soon disappear, leaving a very thin, perfectly homogeneous flat disk that shows a brilliant blue green color when seen on edge. No refractory edge can be made out. This stage is difficult to see. 8. After a few minutes the disk shrinks in diameter and is thrown into rope-like folds around the periphery. 9. Very soon thereafter a refractory edge begins to appear as the folds disappear. 10. Very fine grains presently begin to appear until about 1,400 are formed. Many of these coalesce to form larger grains until only about 600 to 700 remain in the newly formed daughter nucleus. (Further reduction in number may occur during the next few hours.) While the small grains are appearing, the edge of the nucleus becomes more and more refractory until in the new nucleus it is seen as the new nuclear membrane. The steps outlined here require about 28 minutes.

The mitotic events of *Chaos diffluens*, as far as they have been observed, are practically identical with those of the above-mentioned species, except for size.

Correlated histories of individual sense organs and their nerves, as seen in living frog tadpoles. CARL CASKEY SPEIDEL.

In the living frog tadpole it is possible to make daily observations on the same individual nerves and sense organs of the lateral-line for many weeks or months. By suitable operations some sense organs may be deprived of their nerve supply (nerveless organs), and conversely, some aberrant lateral-line branches may be induced to grow without reaching any sense organ (organless nerves).

Prolonged observations of nerveless organs (1 to 21 months) reveal the following: (1) During the first two months in regenerating or growing zones the sense organs are largely independent of their nerve supply. They grow and divide readily. (2) During later months, however, regressive changes of atrophy and degeneration take place. The organs become smaller. Some degenerate and disappear. Occasionally, however, a nerveless organ may persist for more than a year.

Prolonged observations of organless nerves reveal the following: (1) During the first two months they are largely independent of the sense organs. They grow and become provided with both neurilemma and myelin sheaths. (2) During later months, however, regressive changes ensue. The myelin sheath is not maintained on any functionless fiber. It becomes thinner and ultimately disappears. The unmyelinated fiber resulting may then itself degenerate, leaving only a collapsed neurilemma tube.

Thus, the structural integrity of both lateral-line sense organ and nerve fiber is definitely correlated with the successful establishment of a functional relationship between the two.

Sense hairs and orange granules are specialized structures of lateral-line organs. The behavior of both of these under various experimental conditions indicates their relative independence of nerve influence.

Many other histories involving nerve and sense organ relations in wound zones have also been recorded. Illustrative cine-photomicrographs have been made.

The effect of prolonged starvation on the lipids in Phascolosoma gouldii. CHARLES G. WILBER.

It is known that the muscle in vertebrates serves as a storehouse of fats and that during starvation the fats in muscle decrease whereas the fat in various internal organs is not changed. Whether this is true for invertebrates is not known.

In order to throw light on the problem, worms (*Phascolosoma gouldii*) were starved for one month and then the whole worm, the muscle, and the perivisceral fluid respectively were analyzed for phospholipid, for cholesterol, and for fatty acid. These results were compared with the results of similar analyses made on control worms.

It was found that in the whole worm there was a loss of all lipid constituents. In the perivisceral fluid, phospholipid and fatty acid decreased greatly, but cholesterol did not decrease. In the muscle there was an apparent increase in lipid material which can be explained on the basis of absorption of some of the tissue. In muscle the fatty acid is decreased, as is clear from the larger lipocytic coefficient of the muscle of starved worms.

It is concluded that the perivisceral fluid serves as a storehouse of lipid in *Phascolosoma* and that the muscle does not. Moreover, it seems that phospholipid and fatty acid are used during starvation. Whether cholesterol is also used is not certain. *Phascolosoma* differs, therefore, from the vertebrates in the use of phospholipid during starvation and in the fact that muscle is not the important storehouse of fat.

Protoplasmic clotting in isolated muscle fibers. ARTHUR A. WOODWARD.

Isolated muscle fibers provide a material favorably adapted to the quantitative study of protoplasmic clotting. The cut ends form clots which pass in waves over the length of the fiber. The rate of the clotting reaction can be measured and is expressed as mm. of fiber converted into clot per minute. Single fibers are teased from the adductor magnus of *Rana pipiens;* all solutions used are kept at pH 7.1—7.4 with glycine buffer.

In Ringer's solution, used as a standard for comparison, the rate of clot formation is constant for a given fiber and varies only moderately from fiber to fiber within a muscle. The normal rate is about 0.050 mm./min.

Ca ion causes a very rapid clot formation; in this case it is shown that the rate is largely a function of the speed with which Ca diffuses into the end of the fiber, the protoplasm clotting with great rapidity once it is exposed to free Ca ion.

The clotting process is relatively insensitive to pH changes in the region from pH 5 to pH 9; above and below this the rate increases very rapidly. Near the regions in which thrombin is inactivated liquefaction has been observed under certain conditions.

Solutions of crystalline trypsin cause clot formation at a rate averaging about 50 times that of the control. In the absence of Ca, trypsin produces only a slight increase over the control. Crystalline chymotrypsin is much less active than trypsin and also has very little effect in the absence of Ca. Preparations of crude papain cause clot formation at a moderately high rate; addition of glutathione increases the effect to the magnitude of that produced by trypsin. Absence of Ca has no effect on the action of papain.

AUGUST 24

Some aspects of the histology and physiology of luminescence in "railroad worms."
JOHN B. BUCK.

In the Uruguayan "railroad worm," *Phrixothrix,* the lateral photogenic organs are small compact ovoid masses of small dense cells near the posterior edges of the segments somewhat above the spiracular level. The organ is apparently supplied by one trachea ramifying profusely between the cells. There are no end-cells. Large oenocyte-like cells are present near some of the lateral photogenic organs and elsewhere.

In *Phengodes,* a close American relative of *Phrixothrix,* the lateral organs are in the posterior ends of horizontal rolls of tissue which extend along the segments ventral to the spiracles. Light is also emitted along the dorsal posterior edges of most of the segments. Both lateral and dorsal organs apparently consist of loose aggregations of very large oenocyte-like cells without end-cells or special tracheal supply. Similar cells are present in small numbers in parts of the body not regarded as luminous but not in the lateral tissue roll except in the region which emits light. Further evidence is furnished by the observation that the light of *Phengodes* can be seen microscopically to come from clusters of round or oval spots corresponding in shape, position, number, and size to the oencyte-like cells.

The photogenic organ of *Phyrixothrix* is very similar to that in the larval firefly and agrees with the generalization that luminous beetles which produce a lingering glow rather than a short flash, have organs of relatively simple structure without end-cells.

The photogenic organs of *Phengodes* are the simplest yet known in insects and represent the first time that bioluminescence has been ascribed to eonocytes. A corresponding physiological simplicity may be the fact that the light is continuous.

Phengodes dims in the vapor of 10^{-2} and 10^{-3} M. HCN at about the same rate as luminous bacteria, and faster than fireflies.

Effect of caffeine concentration upon retardation of Arbacia development. RALPH HOLT CHENEY.

Sea urchin eggs and sperm were subjected to eight different concentrations of caffeine-in-sea-water for 15 minutes, then mixed for fertilization and the developmental rates in S.W. and S.W.C. were compared with the normal rate of untreated ova and sperm. Observations were made at intervals during a three-day period. Normal time rates were accepted as stated by E.B. Harvey, 1940 (*Biol. Bull.,* **79**, (1) Plate II, photographs 16–32 inclusive).

All eggs utilized in a single experiment were obtained from the same female and all sperm from one male. Eggs and sperm were shed directly into S.W. or S.W.C. prior to mixing. The series of six combinations presented in 1942 (*Biol. Bull.,* **83**) were repeated and extended to a full three day period as follows:—N♀ × N♂, N♀ × C♂, C♀ × N♂, C♀ × C♂, all developed in normal sea water after the original immersion of fifteen minutes after shedding as indicated into S.W. or S.W.C. In the cases of C♀ × N♂ and C♀ × C♂, each was developed also in S.W.C.

Results indicated that the period of immersion (15 min.) in the caffeine concentrations employed prior to mixing the gametes did not render the ova non-fertilizable subsequently nor destroy the ability of the sperm to fertilize. Eggs and/or sperm, however, were not unaffected at least by higher concentrations, since C♀ × C♂ cultures, although they did form the fertilization membrane when mixed and developed in uncaffeinized S.W., the fertilized ova never survived longer than the early cleavage stages.

Plutei developed in normal time and form in all of the six combinations of 0.002 per cent and 0.004 per cent S.W.C. Gametes shed into two per centum S.W.C., in each of the four combinations developed in S.W. formed the F.M. but showed retarded development and in no case reached the pluteus stage before death. In the two combinations developed in S.W.C., the F.M. was not formed. Intermediate percentages used between these extremes of concentration gave intermediate effects indicating that in general, the effects were directly proportional to the concentration of the caffeine.

Other experimental series subjected normally fertilized ova (N♀ × N♂ in S.W.) which had developed to a desired stage of development, to the different concentrations of caffeine-in-S.W. Results here indicated similarly that the retardation effect upon the development time was proportional to the concentration.

Shape changes in the denuded Nereis egg preceding first cleavage. ALBERTA T. JONES.

It is known from Hoadley (1934) that Nereis eggs undergo a series of amoeboid changes prior to first cleavage. Neither the reason for this phenomenon nor the exact pattern followed has been completely described. It is the purpose of this paper to report the principal findings on the pattern of shape changes up to first cleavage in the egg of *Nereis limbata.* The denuded egg was used to eliminate the complications of membrane and external jelly.

Gametes were taken from animals caught the previous night in Eel Pond (Woods Hole) and artificial insemination was carried out. The fertilized eggs were denuded by treatment with alkaline 0.53 molar NaCl solution brought to pH 10.5 by addition of Na_2CO_3. This is the method used by Costello (1939 and 1945). Observations began when the denuded eggs were rinsed free of alkali and the first polar body had formed. Outline drawings of the eggs were made with a camera lucida at three minute intervals. To serve as reference points, the position of polar bodies and oil droplets was indicated.

Hoadley states:—"pulsations of the (Nereis) egg are of two sorts, one of which is quite extensive and results in general distortion of the sphere, and the other of which results in surface irregularities which appear more or less localized." The shape changes discernible in denuded eggs seem to correspond to Hoadley's first category. A consistent pattern of sequences, different from those described by Hoadley for the intact egg, has been found. The sequences include such general distortions as: (1) polar flattening followed by rerounding; (2) elongation in the polar axis followed by rerounding; and, (3) elongation in the equatorial axis followed by formation of the first cleavage plane. The magnitude of these changes, in comparison with the pulsations observed by Hoadley, may be attributed to the absence of jelly mass and membrane.

It may be concluded that shape changes in the denuded Nereis egg prior to first cleavage proceed (1) according to a definite pattern; and (2) always with a particular relation to the polar axis of the egg.

Hormone control of dehydrogenase activity of Crustacean tissues. ELOISE KUNTZ.

Sea water extracts of the sinus glands of *Libinia emarginata, Homarus americanus, Uca pugilator* and *U. pugnax* and similarly prepared extracts of the central nervous system of *Libinia, Homarus* and the arachnoid, *Limulus polyphemus,* were made. These were boiled and centrifuged and the supernatant fluid was used. The extracts were tested for their effect upon dehydrogenase activity of gastric gland and muscle of *Libinia, Homarus* and *Limulus,* which were measured in Thunberg tubes with methylene blue as the hydrogen acceptor.

The effect of sinus gland extract was dependent upon the concentration. Half of a *Libinia* sinus gland doubled the rate of methylene blue reduction, but the reduction rate rapidly fell to slightly above that of the controls with increasing concentrations. Half of a *Uca pugilator* sinus gland also doubled the reduction rate, but activity remained high for concentrations of 2 sinus glands, falling to the level of the controls at 6 sinus glands. Here it remained. *Uca pugnax* showed strong inhibitory action in concentrations of 6 to 12 sinus glands. The character of the curves suggests the possibility of two active substances which vary in relative proportions in different species.

Central nervous system extracts of *Homarus, Libinia* and *Limulus* in concentrations of 0.7 mg. tissue per cc. strongly stimulated dehydrogenase activity. Extracts of other tissues were ineffective at several times this concentration.

Localization of hormone production within the nervous system was demonstrated. In *Homarus* the circumoesophageal ganglia and second ventral ganglion were most effective. The brain and suboesophageal ganglion were ineffective. The remainder of the ventral cord had a relatively weak action. All parts of the circumoesophageal ring of *Limulus* were effective.

An antagonistic action of sinus gland and nervous system extract was demonstrated. The addition of sinus gland hormone to a system stimulated by central nervous system extracts depressed dehydrogenase activity to that of the controls.

A comparative study of cholinesterase activity in normal and genetically deficient strains of Drosophila melanogaster. DR. F. POULSON AND E. J. BOELL.

Cholinesterase activity has been determined in late embryos of several strains of *Drosophila melanogaster* by means of the cartesian diver technique which measures the evolution of CO_2 from Ringer-bicarbonate solution following hydrolysis of acetylcholine in the presence of an atmosphere of 95 per cent N_2 and 5 per cent CO_2. Timed eggs from a stock of the deficiency known as Notch ^{8} crossed to Canton-S wild strain ($N^8/+$) were dechorionated by Slifer's hypochlorite method and classified as normal or Notch-deficient. At 24 hours normals are larvae ready to hatch, while the deficient male embryos are strikingly abnormal and possess a nervous system about three times normal size. The ratio of types is 3 normal: 1 abnormal. Embryos were cut up and placed in divers containing 1 $mm.^3$ Ringer-bicarbonate and 0.5 $mm.^3$ of 1.5 per cent acetylcholine. Although it is possible to carry out measurements on single embryos, two to five embryos per diver were used in most experiments. Readings were made at ten minute intervals for one hour after the divers had reached thermal equilibrium.

A series of four determinations on 24-hour normals gave an average of 12.8 m μ.l. CO_2/embryo/hour. A series of five determinations on Notch ^{8} deficient male embryos gave an average of 34.0 m.μ.l. CO_2/embryo/hour. The cholinesterase activity of Notch embryos is 2.7 times that of normal, which is nearly the same as the volume ratio of Notch/normal nervous systems, 3.3 as determined by planimeter from camera lucida outlines of sections. Thus cholinesterase activity is proportional to volume of nervous tissue. Notch-deficient embryos of other strains have given similar results. Thus the Notch male nervous system while abnormal in size and morphology is biochemically normal with respect to cholinesterase. A first step has been made in studying the rate of increase with development of cholinesterase activity in both normal and deficient embryos. At 18.5 hours the activity of the Notch embryo is 8.3 m.μ.l. CO_2/hour, that of normal 3.8 m.μ.l. CO_2/hour. In the unhatched Notch embryo at 48 hours the activity increases to 51.0 m.μ.l. CO_2/hour.

As checks, cholinesterase activity of unfertilized eggs and methyl butyrase activity of normal and Notch embryos were measured and found to be negligible.

To determine the location of cholinesterase in normal embryos, central nervous systems were dissected out and their cholinesterase activity measured separately from the remaining portion of the embryos. One determination has given a value of 17.0 m. .l./N.S./hour. The value for the remnant is 2.6 m.μ.l./hour. Since the central nervous system makes up not more than one-sixth the embryonic volume the cholinesterase activity there is roughly forty times that in the remnant.

*Possible metabolic and physical chemical factors in the production of the injury potential in spider crab nerve.** A. M. SHANES.

In contrast to frog sciatic nerve, spider crab nerve is permeable to both potassium and chloride ions and to a lesser extent to sodium. The relationship between metabolism and the potentials therefore cannot be the same as in frog nerve which is highly impermeable to chloride and other small anions as well as to sodium. This is confirmed by the following observations: (1) Although 0.002 to 0.0006 M iodoacetate (IAA) produces a continuous slow fall in

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potential in oxygen, 0.02 M pyruvate is unable to counteract this inhibition; (2) pyruvate accentuates IAA inhibition of the post-anoxic recovery of potential; (3) the decline of potential in nitrogen is more rapid than in frog nerve and is not hastened by IAA; (4) glucose does not retard the fall in potential during anoxia and inhibits a recovery in oxygen. The inhibitory effects of pyruvate and glucose may be the result of acid production, for 5 per cent CO_2 lowers the potential in this system in contrast to its effect in frog nerve.

These results provide a basis for understanding some peculiarities of carbohydrate metabolism in spider crabs. For example, blood sugar levels average only 1 mg. per cent, nerve glycogen ranges from 500 to 2,000 mg. per cent wet weight (Kleinholtz, unpublished), and the breakdown of glycogen is reported to be largely to simple sugars as well as to lactic acid.

The potassium content of these fibers is known to be high and the potential is inversely related to the extracellular potassium concentration. Consequently the injury potential is probably a potassium concentration potential as in frog nerve. In yeast (Rothstein and Haege, 1943) potassium retention is stoichiometrically related to hydrogen ions lost to the medium when glucose is assimilated to form reserve carbohydrate. This mechanism may explain the high levels of both glycogen and potassium, and hence the metabolism-potential relationship, in spider crab nerve.

Some effects of tannic acid on osmotic hemolysis. T. H. Wilson and M. H. Jacobs.

Human erythrocytes are less easily hemolyzed in hypotonic solutions of NaCl in the presence of tannic acid than in its absence. The salt solution employed in the present experiments ranged from 0.091 to 0.069 M and those of tannic acid from 1/800 to 1/51,200 per cent. Even at the lowest of these concentrations of tannic acid there was a marked protective effect. In hypotonic solutions of Na_2SO_4 ranging from 0.045 to 0.031 M and with the same concentrations of tannic acid as before, the effect was the exact opposite, hemolysis invariably being increased except at the lowest concentrations of tannic acid. Very similar effects, somewhat complicated by the permeability of the erythrocyte to ammonium salts, were obtained with NH_4Cl and $(NH_4)_2SO_4$, respectively.

In the light of results obtained with molecular films of proteins by Schulman and others, the action of the tannic acid in the chloride solutions might be explained either by a strengthening of the cell surface or by a decrease in its permeability to hemoglobin. Such an action in the case of the sulfate solutions is not necessarily excluded, but it seems to be overshadowed by another effect of a different nature, namely, the decreased permeability to anions produced by tannic acid, described elsewhere by Jacobs, Stewart and Butler. Since swelling of the erythrocyte is known to be opposed by the exchange of bivalent sulfate ions from the outside for univalent anions from the inside, tannic acid, by hindering this exchange, might in this particular case indirectly favor hemolysis, despite its more direct protective effect on the cell surface.

The effect of roentgen radiation on protoplasmic viscosity changes during mitosis. Walter L. Wilson.

Roentgen radiation has a marked effect on the protoplasmic viscosity of the dividing sea-urchin egg. If the eggs, or sperm, or both the eggs and sperm of *Arbacia punctulata* are irradiated before fertilization, then the normal pattern of viscosity change is altered. In the control the viscosity was low shortly after fertilization, then increased to a peak at 15 minutes (23° C.). It remained high for 6–10 minutes and then decreased. This decrease was markedly retarded by irradiation of the sperm or eggs (11,300 r at 6400 r/m), or both the sperm and eggs (5,000 r at 6400 r/m) before fertilization. In these experiments the viscosity remained high two or three times longer than in the controls. In three experiments out of ten in which irradiated eggs were fertilized with normal sperm, the viscosity increased to a value almost twice that of the controls.

Biological specificity and the synthesis of native proteins. D. Wrinch. No abstract submitted.

PAPERS READ BY TITLE

*The effects of massive doses of ergonovine maleate * upon the smaller blood vessels as seen directly with the microscope in the living mammal.*[1] RICHARD G. ABELL.

In these experiments, as in those described above, the blood vessels were studied in transparent 'moat' chambers in rabbits' ears. All injections were of 3.0 mgm., and all were made intravenously. Three mgm. in a rabbit corresponds to 60 mgm. in a man, which is 600 times the clinical intravenous dose. In these experiments the injection of 3.0 mgm. caused complete arteriolar constriction for 3 to 4 minutes. The larger arterioles (80 to 90 microns in diameter) remained narrowed to approximately one-half of their control diameters for 3 to 4 hours. A temporary constriction of the venules to from 0.5 to 0.8 of their control diameters occurred. In addition, this amount of ergonovine caused thicking of leukocytes to the walls of the arterioles, capillaries and venules. The degree of sticking varied widely in different rabbits; in some cases it was slight; in others large numbers of leukocytes stuck to the walls of the capillaries and venules, and emigrated into the surrounding tissue. In one rabbit injections of 3.0 mgm. were followed by the formation of leukocytic emboli, which blocked many of the capillaries and venules and formed thrombi. The reaction was reversible and the thrombi usually disappeared within approximately 4 hours following the injections. This is in accord with the flow toxicity of ergonovine, and its failure to produce gangrene on repeated injections.

As shown by the work of numerous investigators, two other ergot alkaloids, ergotoxine and ergotamine, do produce gangrene on repeated injection. Such gangrene also occurs in ergot poisoning and is due to obliterative endarteritis and thrombosis. The formation of these thrombi is usually attributed to prolonged constriction of the small arteries, and interruption of the blood flow, but this is entirely hypothetical.

In the present experiments thrombi were formed due to the increase in stickiness of the endothelium toward leukocytes and of the leukocytes toward each other.

Perhaps gangrene produced by ergot and its more toxic alkaloids may be caused by a more severe and prolonged reaction of the type described above.

Secretory cells in the branchial epithelium of fishes. GERRIT BEVELANDER.

It was shown by Smith (1930, 1931, 1932) that the osmotic regulation of the body fluids in fresh and salt water teleosts and in elasmobranchs is effected considerably by the extrarenal excretion of salt (NaCl and KCl) under conditions that probably involve considerable osmotic work. It was further inferred that this exchange occurred in the gills. A previous study of the branchial epithelium in an extensive and widely divergent group of fishes (Bevelander (1935), led this writer to conclude that the only specialization occurring in the branchial epithelium of fishes consists in a thicker epithelium in the elasmobranchs than in teleosts and the presence of numerous mucous cells in all species examined. The cells which we described as mucous, were alleged to be "chloride secreting" cells in Anguilla and in some fresh water teleosts, but not in elasmobranchs by Keyes and Willmer (1932).

A re-examination of this problem included the experimental stimulation of secretion of the cells in dispute in representative teleosts and elasmobranchs. These cells were then subjected to a number of histochemical tests and were shown to be positive for mucin. Further, the oral and opercular membranes were also examined and it appears unlikely on the basis of structure that they are concerned with osmotic regulation.

In order to comply with the observed physiological data, the cells which are responsible for extrarenal excretion must be in intimate relation with the blood supply and the external milieu, they must be very extensive to account for the considerable work performed, and finally they must be present in teleosts and elasmobranchs. Our observations reaffirm the absence of any specialized structures; the only cells which comply with the three criteria required are the respiratory epithelial themselves. It is further suggested that the observed conservation of urea in the elasmobranch gills may be affected by the relatively thick respiratory epithelium which covers the gill filaments.

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* 'Ergotrate' (Ergonovine Maleate, U.S.P., Lilly).

[1] This work was aided by a grant made by Eli Lilly and Company to the Department of Anatomy of the University of Pennsylvania Medical School.

A modified Crompton formula for the latent heat of vaporization. ALBERT P. MATHEWS.

The general formula for the latent heat, L, which I have found is

$$L = CR'T \ln_e(d/D) \tag{1}$$

R' is the actual value of the gas constant in the liquid phase, constantly falling as molecular co-aggregation increases with falling temperature. d is the liquid density and D that of the vapor, C is a constant peculiar to the substance, often not far from 2.

The values of C and R' are obtained from the following general formulas which apply to all non-associating substances with the possible exceptions of hydrogen and helium.

$$C = C'(R/R'_c)(L/(L-E))_c \tag{2}$$

$$C' = 1.1292 - (3/8)S + (9/64)S^2 = 0.8792 + ((3/8)S - 0.5)^2 \tag{3}$$

S in (3) is the critical coefficient: RT_c/p_cV_c in which R has its ideal value. S may be computed by (4):

$$S = [(d_mRT_c/Mp_c) + 16((T_c - T)/T_c) - 12((T_c - T)/T_c)^2] \\ /[1 + 5.158((T_c - T)/T_c) - 3.158((T_c - T)/T_c)^2] \tag{4}$$

d_m is the mean density of saturated vapor and liquid at temperature, T; p_c and T_c the critical pressure and temperature.

$$(L/(L-E))_c = 1 + 64R^2/27S^2R'^2_c \tag{5}$$

$L-E$ is the internal latent heat of vaporization. (5) is obtained from (6).

$$((T/p)(dp/dT))_c = (L/E)_c = 1 + (27S^2 R'_c)/64 R^2) \tag{6}$$

$$R'_c/R = (512 - 64S + 216S^2 - 27S^3)/512S \tag{7}$$

R'_T in (1) is obtained from (8).

$$(L/(L-E))_T = R'_T/R'_o = 1 + ((L/(L-E))_c - 1)((9/16)(T/T_c) + 7/16)(T/T_c)^2 \tag{8}$$

$$R'_o = R'_c/(L/(L-E))_c \tag{9}$$

At absolute zero $L/(L-E)$ is 1 and it advances with temperature as co-aggregation diminishes. In the ideal state C, S and $(L/E)_c$ are 1. When S has its highest value of 4 in a normal substance $(L/E)_c$ will be 7.3346 and, when S has its lowest value of 3/8, $(L/E)_c$ will be 4. S may be calculated also from the latent heat of vaporization at any temperature, C' being equal to $(L-E)/RT\ln_e(d/D)$ by (10):

$$S = (8/3)(0.5 + \sqrt{(C' - 0.8792)}) \tag{10}$$

Obtained from (3) above; E being taken, with small error usually, as equal to $p(V-v)$.

Formula (1) above is an easier and more accurate way of computing the latent heat of vaporization than by the thermodynamic equation: $L = (Tdp/dT)(V-v)$. The results by (1) agree usually within 1 per cent with the experimental determinations at the normal boiling point as made by J. H. Mathews and others. The derivation of all the foregoing formulas will be given in the full papers together with examples of application to specific cases and also the general formula for the Cailletet and Mathias law (11):

$$d_m = d_c[1 + (5.158 - 16/S)((T_c - T)/T_c) + (12/S - 3.158)((T_c - T)/T_c)^2] \tag{11}$$

Heat Death. Paul R. Orr.

I. Time-temperature relationships in marine animals.

Temperature as an intrinsic ecological factor which determines, to a great extent, the abundance, life cycle, and distribution of marine organisms, is a well-established fact. However, the duration of exposure required to produce death at each temperature in the effective series has not been taken into consideration. Thus, in order to state accurately the conditions of heat death, it is necessary to plot a curve in which both variables, temperature and time, are represented.

Heat death curves have been plotted for *Uca pugilator, Asterias forbesi, Ophioderma brevispinum, Arbacia punctulata, Nassa obsoleta, Fundulus heteroclitus.*

All of the curves have approximately the same shape. For a relatively slight rise in temperature there is a marked drop in the length of exposure necessary to cause death. This relationship is not one of direct proportionality.

II. Differential response of the entire animal (*Rana pipiens*) and several of its organ systems.

Whether we are dealing with cells or multicellular organs and tissues, or the organism as a whole, we are confronted with the fact that not all of the cells, organs, etc., have the same sensitivity to heat. An animal exposed to excessive heat for a length of time to cause complete loss of excitability might well be pronounced dead, for it never again will show any signs of life as a complete organism. Yet there are parts of the complex animal that are "alive."

The animal as a whole, the tadpole, sciatic nerve, sartorius and gastrocnemius muscles, and heart were separately studied, and curves were plotted for the heat death points of each. The data show that in the adult animal the order of death is: (1) the organism as a whole; (2) the muscular system; (3) heart, and (4) nervous tissue.

All heat death curves plotted are of the same shape, showing a sudden drop followed by a gradual approach to a constant level.

III. The effect of high temperatures on heart rate in *Venus mercenaria.*

In the clam heart (*Venus mercenaria*) we have an automatic mechanism by which the effect of heat can be studied. By subjecting excised hearts to a series of high temperatures and noting the heart rate it was possible to determine the lethal point for each temperature and thus plot a curve showing time/temperature relationship.

For the clam heart the same general type of curve was found as shown in previous studies on marine animals and frogs. That is, there is a point at which the hearts will beat for a relatively long period of time; then as they are subjected to higher temperatures there is a rapid decrease in heart rate, followed by a leveling off to a constant rate.

Penetration glands in tapeworm Onchospheres. W. Malcolm Reid.

Although various types of cystogenous and penetration glands have long been figured and studied as a part of the internal structure of trematode cercariae and miracidia, they have not been recognized in the onchosphere stage of cestodes. A pair of such glands has been found in the fowl cestodes *Raillictina cesticillus* (Molin), and *Choanotaenia infundibulum* (Bloch) and in a herring gull cestode *Hymenolepis* sp. Although these glands may be seen under favorable conditions without special stains, they respond in the same manner to vital stains as do trematode glands, showing up best with Nile blue sulfate and neutral red. The gland stretches to the posterior end of the larva, where it appears to be anchored. The secretion pores are located near the anterior and slightly to the side and above the middle pair of hooks when these hooks are oriented with the points directed anteriorly and downward. The granular contents may be seen to move about as the general contour of the glands is changed by the violent contractions associated with hook movements and at times some of the secretion may be seen exuding from the pores. A single nucleus is located near the middle of each gland, and the two glands are connected by a narrow isthmus near the posterior end.

The nature of the secretion has not been determined but it is possible that it assists the larva in penetration since this granular substance is given off at a time in the life cycle when the six-hooked embryo must break out of the covering membranes of the egg and penetrate the gut of an arthropod intermediate host.

Intensity-duration relation in stimulation by light. F. J. M. SICHEL AND P. B. ARMSTRONG.

The excised sphincter pupillae of many vertebrates will respond by constriction to stimulation by visible light of suitable wave-lengths. In these experiments the *sphincter pupilla* of the eel, *Anguilla rostrata,* was used. The sphincter was excised from small adults, 15 to 18 inches in length.

The sphincter was pinned out, anterior surface uppermost, on white beeswax. It was illuminated for observation by transmitted red light, to which the preparation is insensitive. The source of light for stimulation was a tungsten filament lamp maintained at constant voltage. This was focussed on the preparation obliquely from above. The intensity of the stimulating light was varied by Wratten neutral filters and a neutral wedge. The duration of the stimulating flash was controlled by a shutter manually operated and timed by a stop watch. The criterion of threshold was the smallest contraction visible through a low-power microscope. An eyepiece filar micrometer was used to advantage in determining the threshold stimulus. The preparation was bathed in a Ringert's fluid and permitted to become dark-adapted before each experiment.

The threshold was found to be a function of the duration and of the intensity of the stimulating flash. The intensity-duration relation conforms with Hill's theory of excitation for rectangular stimuli. The chronaxies averaged about 12 seconds, the range being from about 6 seconds to 20 seconds. In terms of the reciprocity law this would mean that the law holds reasonably well for flashes shorter than, say, 10 seconds. At longer durations the deviation is, in direction and amount, what would be expected on the basis of Hill's equation for excitation. There is a definite rheobase, or minimal intensity of the stimulating flash below which excitation is never produced, even for very long exposure times.

The pattern of the intrinsic palmar musculature. WILLIAM L. STRAUS, JR.

The intrinsic palmar musculature of tetrapod vertebrates comprises two fundamental series: (1) a superficial, arising from *fascia or tendon,* and showing variable tendency toward stratification, and (2) a deep, arising from *bone* and always arranged in two layers separated by the deep palmar nerves and vessels. Between the two series lies the mid-palmar space.

In urodeles (*Necturus maculosus, Cryptobranchus alleghaniensis*), the superficial series is a single layer (flexores breves superficiales) arising from the dorsum of the long flexor tendon. The deep series is composed of a superficial (contrahentes or adductores) and a deep (flexores breves profundi, intermetacarpales, interphalangeus III?; in *Cryptobranchus* also flexores breves minimi) layer.

In lizards (*Sceloporus spinosus, Ctenosaura similis*), the superficial series tends to form two layers—a superficial (flexores breves superficiales, marginal abductors), arising from the transverse carpal ligament, and a deep (lumbricales), arising from the long flexor tendon; in *Sceloporus,* however, such lamination is incomplete, for fibers of the superficial layer also arise from the long flexor tendon. The deep series again exhibits superficial (contrahentes) and deep (flexores breves profundi) layers.

In mammals (*Didelphis virginiana, Macaca mulatta, Homo*), the superficial series forms two distinct layers—a superficial (abductor pollicis brevis, flexor pollicis brevis, opponens pollicis?, palmaris brevis, flexor V brevis, abductor V; in *Didelphis* also a flexor brevis manus), largely from palmar aponeurosis and transverse carpal ligament, and a deep (lumbricales), from the deep long flexor tendon—separated by the superficial palmar vessels and nerves. The deep series again has superficial (contrahentes; only adductor pollicis in man) and deep (interossei, opponens V) layers.

Muscular homologies, at least between vertebrate classes, cannot be reasonably extended beyond comparison of entire palmar layers. Direct homology of individual muscle units is profitless and probably invalid.

The toxicity of a mixture of high molecular alkyl-dimethyl-benzyl ammonium chlorides to Fundulus. CHARLES H. TAFT.

The mixtures of high molecular alkyl-dimethyl-benzyl ammonium chlorides used is sold by the Winthrop Chemical Company under the trade name Zephiran Chloride * for use as an antiseptic or disinfectant.

Taft and Strandtmann (1945. *Fed. Proc.,* **4**: 136) showed that under laboratory conditions this material is an efficient larvicide for the mosquito Culex quinquefasciatus and Aedes aegypti in dilutions up to 1:250,000. It seems desirable to determine its toxicity to some of the animals it might be brought in contact with if used for this purpose. Taft (1946. *Texas Rpts. on Biol. and Med.,* **4**: 25) has reported its toxicity for various invertebrates.

To determine the toxicity by injection fundulus were injected intraperitoneally with different doses of one per cent solution; 0.25 cc. killed 17 out of 22, 0.05 cc. killed 15 out of 17 while 0.1 cc. killed 24 out of 24 fundulus. When these fish died they were darker than the controls and in many of them the abdomen was red about the site of injection. When the abdomen was opened there was frequently a greenish fluid present and the viscera had the appearance of having been cooked. The liver, gall bladder, heart, kidneys, and gills appeared normal.

Other fundulus were placed in finger bowls containing 225 cc. aerated sea water with different concentrations of the drug. When the fish were placed in dilutions of from approximately 1:2,500 to 1:100,000 all the fish died in from 35 to 105 minutes. On autopsy there were no significant gross changes. A dilution of 1:225,000 killed 25 per cent of the fish while 1:500,000 did not kill any of the fish exposed to it.

To determine the effects of longer exposure to the drug several fundulus were placed in battery jars in aerated sea water solution of from 1:100,000 to 1:400,000 and observed at the end of 24 hours. All the fish exposed to 1:100,000 and 1:200,000 were found dead. Twenty-five per cent of those exposed to 1:300,000 died while the 1:400,000 solution failed to kill any fish. It is evident that the effective range of this drug when employed as mosquito larvicide might be deleterious to fundulus.

Further evidence of polypoidy in the conjugation of green and colorless Paramecium bursaria. RALPH WICHTERMAN.

In a study of the time-relations of the nuclear events in living and Feulgen-stained preparations through conjugation, instances of polyploidy were encountered. Polyploidy was first recorded in Paramecium by Chen (1940, *Proc. Nat. Acad. Sci.* **V**: 26) for *P. bursaria* and this represents the second report of the phenomenon. Pure-line races of the colorless (255) and green (B9) paramecia were mated. The individuals of each race have well-defined micronuclei of approximately equal size.

The three pregamic divisions were found to be remarkably constant in respect to time and micronuclear behavior at a given temperature. However, in the cytological examination of many hundreds of joined pairs, approximately 2 per cent were observed in which the micronuclear behavior resulted in the polyploid conditions only after the pregamic divisions. The crucial stage where polyploidy occurs is found during the period of pronuclear transfer, approximately 16–18 hours after the animals have been mated. It follows the third suggestion made by Chen in accounting for polyploidy; namely, the failure of a migratory pronucleus in one of the conjugants to migrate to the other conjugant. The result is an individual with one small pronucleus (the "stationary") which is haploid, and the conjugant with three pronuclei (two "migratory" and one "stationary") which fuse and form a larger triploid synkaryon.

What is the fate of each nuclear body that is now comparable to the normal synkaryon? The subsequent micronuclear stages show a conspicuous and persisting size difference in all later stages and hence are recognized easily. In the haploid conjugant, late anaphase stages (comparable to postgamic ones) measure 10.8 μ in length and are very narrow; similar stages in the triploid co-conjugant measure 27 μ in length and are proportionately wider. Their division products measure 8 μ in the haploid and 15.5 μ in the triploid individuals respectively.

While polyploidy occurs in only 2 per cent of the cases in this material, it nevertheless creates variation in micronuclear composition and is therefore of evolutionary significance.

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* Kindly furnished by the Winthrop Chemical Company.

The Lipids in Pelomyxa carolinensis. CHARLES G. WILBER.

In 1942 the author demonstrated that the cytoplasm of *Pelomyxa carolinensis* contains lipid material, that this lipid comes from digested food, and that it is composed of a high proportion of fatty acid. In the latter respect the stored fat differs from that in *Amoeba proteus* in which fat is stored in the neutral form. In the previous work, Nile blue sulfate was used to distinguish neutral fat from fatty acid. This dye has been criticized as a reagent for fat tests. Consequently it seemed desirable to use specific chemical procedures to ascertain the nature of the lipid material in *Pelomyxa*.

Ninety mg. (wet weight) of pelomyxae were thoroughly washed in boiled culture fluid. By repeated centrifugation the cells were broken up and then the lipids were extracted in hot alcohol. The quantities of phospholipid, cholesterol, and fatty acid were ascertained by the Bloor method. It was found that in the amount of cellular material used there was no measurable phospholipid or cholesterol. The total weight of fatty acid in 54 mg. of cells was 2.05 mg. or 3.8 per cent fatty acid.

These results are in agreement with the results previously obtained using Nile blue sulfate. It seems that in *Pelomyxa carolinensis* the lipid material occurs chiefly as fatty acid and that the amount of other lipids is very small.

The presence of lipase in Pelomyxa carolinensis. CHARLES G. WILBER.

The digestion of fat in rhizopods has been demonstrated by several investigators. Moreover, it has been shown that the fats digested are incorporated into the cytoplasm. However, none of the investigations so far has given direct evidence for the presence of lipase in the cytoplasm of rhizopods.

Pelomyxae were starved and then ground up in a drop of water. A drop of this solution was added to a drop of 0.2 per cent emulsion of castor or olive oil and a drop of pure water was added to another drop of the emulsion as a control. After 30 minutes both drops were treated with hydroxylamine hydrochloride and potassium hydroxide. Then after acidification each drop was treated with 1 per cent ferric chloride solution. In each case a violent brown color was produced in the control, whereas no color was produced in the drop containing the ground up pelomyxae.

The above reaction is a test for esters. Lipases are known to be ester ferments. Since the oil emulsions mixed with ground pelomyxae did not give the characteristic ester reaction, it can be concluded that the esters were broken down by something in the cytoplasm. We therefore have direct evidence for the presence of lipase in *Pelomyxae carolinensis.*

PAPERS PRESENTED AT THE MEETING OF THE SOCIETY OF GENERAL PHYSIOLOGISTS

September 5 and September 6

First Session—S. C. Brooks, Chairman

The effect of cold on capillary permeability and fluid movement in the frog. Ellen Brown, M.D.* and Eugene M. Landis, M.D.

Micro-manipulative methods were used to study the relationship between capillary blood pressure and the rate of fluid movement through the walls of single capillaries in the frog's mesentery (a) at ordinary room temperatures of 22.5° to 25.5° C. and (b) when the mesentery was cooled to between $-2°$ and $+2°$ C. Cooling the mesentery decreased capillary permeability, reduced the observed rates of filtration and increased the observed rates of absorption. The filtration constant of the capillary wall was reduced from the control value of 0.0070 μ^3/μ^2/sec./cm. water pressure to 0.0019, a decrease of 73 per cent. The effective osmotic pressure of the blood within the capillaries was elevated from 10.5 to 13.8 cm. water, an increase of 31 per cent.

Four possible causes for the increase in apparent or effective osmotic pressure were considered. (1) An increase of *absolute colloid osmotic pressure* was excluded because plasma protein concentrations, calculated from specific gravities of plasma samples, were the same in the two series of frogs. (2) An increase in *effective colloid osmotic pressure* due to greater retention of plasma protein during cooling could also be excluded because the control experiments showed that plasma proteins were already retained completely, or almost completely, even at room temperature. (3) It is possible, however, that the *effective non-protein osmotic pressure* might rise if the passage of smaller molecules, e.g., glucose, amino acids, urea or certain electrolytes, was impeded more than that of water as the permeability of the capillary wall decreased during chilling. (4) *Thermosmosis* might also be responsible because relatively warm blood was circulating through capillaries surrounded by cooler tissues. Studies are in progress to determine whether or not this factor modifies the movement of fluid through membranes *in vitro* using a schema which simulates the conditions existing *in vivo*.

The effects of cold on the capillaries of the frog differ from those observed in mammalian capillaries because the former become less permeable at 2° C., whereas the latter become more permeable as temperature falls below 10° C. However, actual freezing of the frog's capillaries at temperatures of $-5°$ to $-10°$ C. increased capillary permeability conspicuously, as shown by the appearance of stasis during thawing. If the duration of actual freezing was brief this stasis usually disappeared within a few minutes as the capillary wall regained its normal relative impermeability to protein.

Bubble formation within single cells.† E. Newton Harvey, K. W. Cooper, A. H. Whiteley, D. C. Pease, and W. D. McElroy.

Normal living isolated cells (Amoeba sp., *Chaos chaos,* Paramoecium, Arbacia and Asterias eggs and Nitella) do not form internal gas bubbles if saturated with nitrogen gas at 80 to 120 atmospheres pressure and then suddenly decompressed. Bubbles may form on the outside of the cells due to contamination with gas nuclei (minute gas phases sticking to hydrophobic spots). Cells which have been killed by chloroform or formalin likewise form no bubbles within but sometimes spontaneously dead cells or those previously injured by twisting before subjection to the high gas pressures do form bubbles within after decompression. A living Nitella cell just after decompression from high gas pressures and still free of bubbles, will immediately form a bubble inside if the cell wall is gently pinched (not enough to penetrate the wall) or twisted. Such bubbles are believed to result from local decreased tensions that tear the liquid, forming a space or cavity (a vapor phase) into which gas diffuses, forming a gas nucleus that immediately grows to a bubble. Such cavities can form inside or outside of cells even at atmospheric pressures. They are believed to be formed during muscular exercise in man, when the incidence of aviator's bends is greatly increased.

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* Research Fellow in Physiology, Commonwealth Fund.

† Part of the work described in this Abstract was done under a contract recommended by the Committee on Medical Research between the Office of Research and Development and Princeton University.

The action of various cations of muscle protoplasm. L. V. Heilbrunn and F. J. Wiercinski.

There are two ways to study the colloidal behavior of muscle protoplasm. One way is to isolate pure proteins and follow their reactions in test tubes; the other way is to subject the protoplasm itself to reagents and observe the results. In our studies, we injected solutions of various salts into the interior of isolated muscle fibers of the frog. We then noted the degree of shortening of the constituents of the muscle. With the aid of a micrometer eyepiece, we were able to determine the effect of the injections on the length of the fiber. In numerous experiments we found that rather dilute calcium chloride solutions invariably caused an immediate and pronounced shortening of the protoplasmic constituents of the muscle. On the other hand, potassium and sodium chloride had very little effect. Even when injected in concentrations isotonic with the muscle, they ordinarily caused no shortening whatsoever. Rarely, a shortening did follow injection of isotonic sodium or potassium chloride. This we believe was due to the release of calcium ion. Magnesium ion likewise causes no shortening of the protoplasmic constituents. Barium acts like calcium. The results support the calcium ion theory of stimulation and they are opposed to Szent-Györgyi's opinion that potassium is the ion primarily responsible for the contraction of muscle.

Further observations on an oligodynamic action of copper and mercury on erythrocytes. M. H. Jacobs and Dorothy R. Stewart.

The specific effect of copper in decreasing the permeability of erythrocytes to glycerol seems to be absent in all species whose erythrocytes show a low degree of permeability to this solute. It is also lacking in a number which show a very high permeability both to glycerol and to other hydrophilic solutes of comparable molecular volume. It has so far been found only in those species whose erythrocytes show a disproportionately great permeability to glycerol, thus suggesting that some special mechanism of penetration may be involved, which is reversibly inactivated by copper. This generalization is supported by the behavior of the erythrocytes of a number of birds in which the specific permeability to glycerol is particularly great.

The effects of $HgCl_2$ in some ways resemble and in others differ from those of $CuCl_2$. One of the most important differences is that $HgCl_2$ forms a double salt with NaCl, and its activity is therefore greatly reduced by the presence of any considerable quantities of the latter salt. A second difference is that $HgCl_2$ readily enters the erythrocyte, while $CuCl_2$ does not. These two fundamental differences are responsible for a number of secondary ones.

That copper may hinder the escape of glycerol from human erythrocytes, as well as its entrance into them, is suggested by the following experiment. To a suspension of erythrocytes in an isotonic salt solution, small amounts of copper and of concentrated glycerol are added, and the resulting mixture is then diluted with a properly chosen hypotonic salt solution. If the copper is added before the glycerol, it decreases hemolysis by preventing the entrance of glycerol into the cells. If the copper is added 30 seconds or more after the glycerol, it increases hemolysis by preventing the escape of the glycerol that has entered the cells.

The prolytic loss of K from red cells. Eric Ponder.

The prolytic loss of *K*, i.e., the loss of *K* which takes place from red cells exposed to hypolytic concentrations of lysin, has been measured by means of the flame photometer in systems containing distearyl lecithin, sodium taurocholate, sodium tetradecyl sulfate, saponin, and digitonin. The lysins are added in various concentrations to washed red cells from heparinised human blood, and the K in the supernatant fluids is determined after various intervals of time at various temperatures. This prolytic loss of K, K_p, is compared in every experiment with the loss K_s into standard systems containing one per cent NaCl alone, without lysin.

The losses K_p and K_s increase with time, so that new steady states are approached logarithmically. The values of K_p which correspond to the new steady state depends on the lysin used, being greatest with taurocholate and smallest with powerful lysins such as digitonin (confirming an observation of Davson and Danielli). The extent and course of the K losses seem to have no simple relation to the prolytic phenomenon of the disk-sphere transformation.

Just as the prolytic loss of K occurs without the loss of any Hb, so in concentrations of lysin sufficient to produce hemolysis the loss of K, expressed as a percentage of the total red cell K, increases much more rapidly with lysin concentration than does the loss of Hb, expressed as a percentage of the total Hb. The explanation of these relations depends on whether the loss of K is treated as being all-or-none in the case of the individual cell, or as being the result of the loss of part of the K from all the cells. This point has yet to be decided.

SECOND SESSION—L. MICHAELIS, CHAIRMAN

Effect of fluoroacetate on the metabolism of baker's yeast. E. S. GUZMAN BARRON AND GEORGE KALNITSKY.

Among the organic halogen compounds, those containing fluorine occupy a special position regarding their chemical and physiological properties. Because of the high value of the energy of the C–F bond and of the electro-negativity of F, the introduction of F into the C atom produces a greater stability, specially in alyphatic compounds. This is shown on measuring the rate of combination of cysteine with halogen acetates. At 23°, half-reaction with iodoacetate took place in 4.4 minutes; with bromoacetate in 6.2 minutes; with chloroacetate, in 125 minutes. With fluoroacetate it did not react at all. There is a certain relationship between the rates of reaction and the bond-energy values of the C-halogen bonds as well as the electronegativity values of the halogens. On studying the effect of these halogen acids on the rate of oxidation of acetate by baker's yeast it was found that 0.001 M of fluoroacetate inhibited it 90 per cent; bromoacetate, 17 per cent; iodoacetate, chloroacetate, and trifluoroacetate, none at all. On comparing the interatomic distances between C and the halogen it can be seen that the C–F bond with a distance of 1.41 Å approaches most closely the distance of the C–H bond, 1.09 Å. By increasing the size of the fluoroacetate molecule through the replacement of the other two hydrogens with fluorine (trifluoroacetate) the inhibiting effect was destroyed. This inhibition is a substrate competitive inhibition, the fluoroacetate occupyng the place of acetate in the protein moiety of the acetate metabolism enzyme. Increase of the length of the molecule as in fluoropropionate, fluorobutyrate, and fluorocrotonate destroyed the inhibition. Inhibition was partially reversed on addition of large amounts of acetate (0.08 M). Inhibition occurs in the first step of acetate metabolism, namely, condensation with oxaloacetate to give citrate. The formation of citrate from acetate was completely inhibited with 0.005 M fluoroacetate. In the presence of ethanol, the rate of O_2 uptake was not affected by fluoroacetate up to 42 per cent of the total O_2 uptake, when the inhibitory effect appeared. This is indication that ethanol oxidation occurs in three successive steps:·oxidation of ethanol to aldehyde; and of aldehyde to acetate, both unaffected by fluoroacetate; and oxidation of acetate, inhibited by it. At the end of the experiment there were in the control 1572 cmm. O_2 used, and 150 cmm. of acetate formed from 940 cmm. of ethanol; in the presence of fluoroacetate there were 975 cmm. O_2 used and 890 cmm. of acetate formed.

The effect of sodium azide on Paramecium calkinski. E. J. BOELL.

Sodium azide is generally regarded as an inhibitor of respiration by virtue of its inactivation of cytochrome oxidase. In *Paramecium calkinsi*, experiments have shown that this compound, in a concentration of 0.001 to 0.01 molar, reversibly depresses respiratory activity by 50 to 60 per cent. Under certain circumstances, however, azide instead of inhibiting respiration serves as a powerful respiratory stimulant. The stimulating effect of azide seems to depend primarily upon the pH of the medium. For example, a 0.01 molar solution of NaN_3 at pH 6.02 will depress respiration to a value about 30 per cent of normal; at pH 6.24 respiration is 70 per cent of normal, while at pH 6.59 the same concentration of azide stimulates respiration of 238 per cent of normal. Calculation of the hydrazoic acid concentration at these pH values shows that the effect produced depends, within certain limits, upon the concentration of undissociated HN_3.

A study has been made of the mechanism of azide stimulation. It has been found that the respiratory quotient of normal animals averages 0.99; that of animals in the presence of a stimulating dose of azide averages 1.05. The increased oxidation thus involves the metabolism of organic substrate. It is also apparently mediated by the normal enzymic mechanisms for it is sensitive to cyanide. Carbon monoxide, however, exerts only a slightly depressing effect.

The metabolism of Paramecia under normal circumstances is accompanied by the production of large quantities of ammonia nitrogen. On the assumption that such ammonia production represents protein breakdown, approximately 75 per cent of the total oxygen consumption of control animals can be accounted for in this way. Although Paramecia treated with azide show increased ammonia production, only 22 per cent of the extra oxygen uptake induced by azide can be accounted for as protein breakdown with ammonia as the end product.

In addition to the effects already noted, azide interferes with the ability of *Paramecium calkinsi* to maintain normal water balance. The activity of the contractile vacuoles is greatly reduced and supernumerary vacuoles are frequently formed.

The oxygen consumption concerned with growth in bacterium coli. KENNETH FISHER. No abstract submitted.

Enzymatic acetylation and the coenzyme of acetylation. FRITZ LIPMANN.

The mechanism of enzymatic acetylation of aromatic amines has been studied in pigeon liver homogenates and extracts (Lipmann, F., 1945. *J. Biol. Chem.*, **160**: 173). In this enzymatic system the condensation of an aromatic amine, like sulfanilamide, with acetate, is effected through a transfer of phosphate bond energy from adenylprophosphate. (Cf., Nachmansohn, D. and Machado, A. L., 1943. *J. Neurophysiology,* **6**: 397 for a similar system of choline acetylation in brain).

A heat stable and dialysable coenzyme was recently found necessary in this reaction, besides the energy donor adenylpyrophosphate. The characterization of this new coenzyme is now in progress in this laboratory in collaboration with Dr. Nathan O. Kaplan. We find the same coenzyme necessary to complement dialyzed brain extracts for acetylation of choline, although the brain enzyme is specific for choline and the liver enzyme specific for amines. The coenzyme is present in largest amounts in brain, liver, and kidney. Appreciable amounts are present in all tissues tested, including carcinoma. Therefore its action must be a very general one and probably not merely restricted to acetylation.

The coenzyme is destroyed by intestinal phosphatase with liberation of phosphate. It is inactivated by a rather general tissue enzyme without liberation of phosphate. The link attacked by the latter enzyme is unknown. The compound follows the general pattern of nucleotide precipitation. Our most active preparations showed sporadic crystals on microscopic examination. This quite uniform fraction contained adenine, ribose, and phosphate in the proportion 1 to 1 to 2. Acid hydrolysis showed the second phosphate not to be in pyrophosphate linkage. If we assume the presence of some crystals to indicate near purity, which it not necessarily does, then the content of approximately 50 per cent of adenylic acid in our best preparations should mean the coenzyme to be of dinucleotide structure. Therein the adenylic acid should be linked through the second phosphate to an as yet unidentified part. Electrotitration and cleavage experiments seem to support the outlined constitution.

Penetration and action of cholinesterase inhibitors. DAVID NACHANSOHN. No abstract submitted.

The metamorphosis of visual systems in amphibia. GEORGE WALD.

In the rods of the vertebrate retina two visual systems are found. One is based upon the red photosensitive pigment rhodopsin, engaged in a cycle with vitamin A_1; the other involves the purple photopigment, porphyropsin, bound in a similar cycle with vitamin A_2.

The porphyropsin system appears to be the more primitive in vertebrate evolution. The cyclostome, *Petromyzon marinus,* possesses only this system. The same is true of all types of freshwater fish so far examined.

Vertebrates have followed two pathways out of fresh water, one into the sea, the other to land. Both have led them to the use of vitamin A_1 in vision. Thus all marine fishes which have been examined, with the single exception of certain Labridae, have the rhodopsin system alone; so also do all the birds and mammals investigated.

Interpolated between freshwater and marine fishes are euryhaline forms, which can exist as adults in either environment. Among them, the salmons and the "freshwater" eel have mixtures of the rhodopsin and porphyropsin systems; while the alewife and white perch have only the latter. In all these forms the visual system is predominantly or exclusively that normally associated with the environment in which the fish develops embryonically, and is relatively independent of the environment in which it is found as an adult.

Interpolated between freshwater fishes and true land vertebrates are the amphibia. Their life histories for the most part are closely analogous with those of euryhaline fishes, amphibian migrations to land replacing fish migrations into the sea.

Adult frogs possess the rhodopsin system and vitamin A_1 alone. The tadpole of the common bullfrog, *Rana catesbiana,* however, has exclusively the porphyropsin-vitamin A_2 system just prior to metamorphosis. During metamorphosis it transfers completely to the rhodopsin system, which is found alone in the new emerged frog. Partly metamorphosed animals have mixtures of both systems, such as have been found otherwise only in euryhaline fishes.

The common newt, *Triturus viridescens,* begins its life as a gilled larva in fresh water. After several months it metamorphoses to the land-living red eft; then after 1–2 years of growth it undergoes a second metamorphosis to the sexually mature newt, returning to the water for the remainder of its life. The eye of the red eft contains a mixture of vitamins A_1 and A_2, predominantly the former; while that of the water-phase adult presents just the reverse proportions of both vitamins. This is a change opposite in direction to that in the frog, but associated in the same way with the chemical metamorphosis of visual systems.

Amphibia, therefore, like euryhaline fishes possess as a group both the rhodopsin and porphyropsin systems; but in amphibia these systems succeed one another as the animal goes through its basic metamorphoses.

THIRD SESSION—J. H. BODINE, CHAIRMAN

X-ray effects in mixtures of compounds. RUBERT S. ANDERSON.

It has been reported previously that ascorbic acid, as shown by experiments in plasma, has a preferential ability to react with the materials produced in water by x-rays. Much of the ascorbic acid reaction is not observed in irradiated muscle. Non-uniform distribution of the ascorbic acid in muscle would tend to make the observed result too low. Another possibility is that other compounds are present in muscle which take some of the reactive material away from the ascorbic acid.

Evidence has been obtained that the ascorbic acid reaction consists in part of a reversible oxidation, presumably to dehydroascorbic acid. When present during the irradiation, glutathione and cysteine gave substantial, although variable, protection of ascorbic acid against x-rays. Alanine was much less effective, suggesting that the sulfhydryl grouping is largely responsible, whether it is a true competitive protection or a reversal of oxidation. Glutathione and cysteine and possibly protein sulfhydryl groups could thus account for a part of the protective effect of muscle on ascorbic acid.

There is no evidence that the destruction of a small amount of these compounds would damage a cell. However, the work shows that, in principle, a compound through which the water reaction might damage the cell could exist.

Ascorbic acid, glutathione and cysteine partially protect pepsin from the inactivating effect of x-rays. Alanine is much less active.

If at least a part of the reaction in water is distributed randomly throughout the proteins of the cell and nucleus, the occasional loss of a molecule or two from compounds represented by hundreds of molecules need have little effect on the cell although the products formed, such as denatured proteins, might secondarily be harmful to the cell. However, if there are in the cell or nucleus some thousands of different protein compounds or structures each form of which is essential to the cell and each one of which is represented by but one or two molecules or particles, then randomly distributed products of irradiated water might destroy one of these entities and so damage or kill the cell. This is essentially the argument used by Lea in reaching the conclusion that his theory led to the expectation of gene and chromosomal effects from irradiation.

Electrical studies of acetylcholine and choline esterase. T. C. BARNES.

Acetylcholine passes through the thin oil layer of a bubble of guaiacol-resin-cholesterol so rapidly that the spike potential must be recorded by an oscillograph. First the acetylcholine produces a negative phase boundary potential on one side of the oil layer but on reaching the opposite side of the oil a new potential is established which produces the descending part of the spike (no esterase required). At the suggestion of Osterhout, solutions were shaken 5 hrs. with guaiacol with these results: oil with saline 5×10^{-7} mhos; same with 0.002 M acetylcholine 35×10^{-7} mhos (conductivity was determined of oil separated from aqueous solution). Applying the Nernst equation, 0.058 times log conductivity difference (6) gives 40 mv. (observed phase boundary potential). At the suggestion of Loewi, tetramethylammonium iodide was found to give no potential on nitrobenzene but 0.05 per cent gave 25 mv. negative on guaiacol. The type of oil and not the tertiary or quarternary nature of the compound determines electrogenic effects. Thus prostigmine produces 85 mv. negative on guaiacol compared with 35 mv. generated by acetylcholine (both 0.002 M). Prostigmine inhibits the cord in spasticity by flooding with high negative potential which may also act as a stimulus on muscle in myasthenia. Dialantin and phenobarbital produce positive potential (20 mv. at concentration of 0.05 per cent) which probably neutralizes the excess negativity of acetylcholine in the brain in epilepsy. Lyovac plasma reduces the phase boundary potential of 0.05 per cent acetylcholine from 35 to 15 mv. (residual potential is produced by choline). Potential of benzyol choline is destroyed by serum and part of the mecholyl potential by one per cent ground cat brain. Eserine and DFP preserve the potential of acetylcholine in the oil-cell in the same manner as in the nerve.

One per cent DFP increases the specific conductance of guaiacol 100 per cent which explains part of its blocking action on nerve and muscle.

The action-current in cholinergic nerve is probably a phase-boundary potential of acetylcholine (sympathin is the electrogenic amine in adrenergic nerve).

Two schools of thought in electrophysiological theory. R. BEUTNER AND T. C. BARNES.

The older school, entrenched as the hypothesis of sieve membranes retaining negative but not positive ions, explains everything but solves no problems. The newer school omits hypotheses and proposes searches for electrogenic materials in tissues by setting up artificial battery systems composed of lipoid layers (oils) inserted between aqueous salt solutions. Some of these resemble analogous battery systems containing a tissue in place of the lipoid. One type of system studied is :—concentrated saline/tissue or lipoid (oil)/diluted saline +. Tissue, in such a set-up, may produce the maximum e.m.f. of 58 millivolts if the concentrated solution is 1/10 mol.; the diluted one, 1/100 mol. Only few oils show such an effect, as e.g., fatty acid dissolved in a phenol-derivative, but not neutral fats, gelatin, etc.

The production of bio-electricity does not depend on such aqueous salt concentrations but on metabolic processes in tissues, chiefly oxidation. A search for suitable electrogenic systems has led to the following one (Beutner, Loznerea, 1930) :—saline/reduced substance e.g., a higher alcohol or lower fatty acid as in dying tissue/oxidized substance e.g., corresponding acid or corresponding higher fatty acid as in respiring tissue saline +. For the action current one possible electrogenic substance is acetylcholine since even dilute solutions produce an e.m.f. in contact with oils in a system such as: + saline without addition/oil/saline with acetylcholine added 1 : 100,000 to 1 : several million—(Beutner and Barnes, 1941).

Tissue extract can be used in the place of the oil, also frog's nerve by the Netter technique. Adrenergic amines produce similar negative potentials but on different oils which are inactive in contact with choline esters. A difference in chemical composition may therefore be responsible for the specific function of cholinergic and adrenergic fibers. The rapid disappearance of the negative potential, which occurs even in the absence of choline esterase, may be explained by a penetration of acetylcholine through a thin lipoid layer (membrane) creating a potential difference in the opposite direction on the other side. Physico-chemical studies are not needed for the search for electrogenic substances, but when performed on oil cells, they show the existence of phase boundary potentials depending on electrolyte distribution; the charged pore theory fails to explain the phenomena and is contradictory.

The frequency of x-ray-induced chromated breaks in Tradescantia as modified by near infrared radiation. C. P. SWANSON AND ALEXANDER HOLLAENDER.

The frequency of x-ray-induced chromatid breaks in Tradescantia can be significantly increased by treatment of the inflorescences with near infrared radiation. Pretreatment with near infrared radiation for seven hours prior to x-radiation inceased the frequency of single deletions, double (isochromatid) deletions, and translocations between and within chromosomes; post-treatment increased only single deletions and translocations. A delay of 21 hours between treatment with infrared and x-rays did not appreciably decrease the effectiveness of the infrared, suggesting that the changes within the cell induced by the infrared were of a relatively permanent nature. At the present time, the nature of the effect of infrared is not clearly understood.

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A STRONGLY INTERSEXUAL FEMALE IN HABROBRACON

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In the parasitic wasp *Habrobracon juglandis* (Ashmead), diploid males have never shown any tendency toward intersexuality; they are as definitely male as their normal haploid brothers. When a "diploid male with female genitalia" was found, it was therefore regarded with especial interest. The specimen, designated freak 994, developing from a heavily x-rayed (29,300 *r*) egg, occurred among the offspring of a treated wild type (stock 33) female crossed with an untreated lemon honey male (Experiment by A. R. Whiting, 1945).

Freak 994 shows the heterozygous condition of the semidominant body color gene lemon inherited from its father. (Note light base of antennae in Figure 1.) The number of its antennal segments and its large ocelli are male characteristics. It was to be expected, therefore, that male reproductive reactions would occur. Several tests at different times failed to evince any response toward females although the specimen appeared healthy, drank honey water and lived for several days until fixed in Carnoy fluid. Since it likewise failed to give any response (female) to caterpillars, its indifference was probably not due to its sex type but to some unknown factor.

Because of the small "feminized" genitalia on the "male" body, freak 994 was at first recorded as a "diploid gynandroid male." Gynandroids, however, have always been haploids. They are mosaic males in which the two sexually different types of male tissue react in a complementary way to feminize the external genitalia (Whiting, Greb, and Speicher, 1934). Their mosaicism is shown by their asymmetry, not only in body color, in number of antennal segments, in mutant traits, and often in wing length, but especially in the external genitalia which are a mixture of normal male and feminized male structures with much reduplication and irregularity. In freak 994 there are no male genital structures and the female genitalia, consisting of a pair of sensory gonapophyses with no visible sting, are symmetrical and larger than in gynandroids. They are much smaller, however, than the female genitalia found in gynanders which are male-female mosaics with clearly separated male and female regions. That freak 994 is not a sex mosaic is shown by its symmetry in body coloration, in antennal flagella with nineteen segments in each and in length of wings and legs.

Two types of intersexes have hitherto been reported in Habrobracon. (1) Gynoid, dependent upon a single mutant gene, is a weakly intersexual male, functioning normally as a male, but having certain external traits, including antennae, feminized. (2) Nine intersexual females were reported (Whiting, 1943) occurring in a single fraternity. "Superficially, these appear to be the reverse of the gynoid males, being more masculine anteriorly, feminine posteriorly." They resemble freak 994 in head and thorax and in the anterior part of the abdomen which are altogether like those of the male. In the posterior region, however, the sclerites are thickened, there is a normal sting and the sensory reproductive appendages are of full length characteristic of the female. "The nine intersexual females must be regarded as more strongly intersexual than gynoid males since antennae, ocelli and instincts are completely sex reversed." Freak 994 is an intersexual female, comparable to these nine but still more strongly intersexual because of greater restriction of the "female" region and reduction of the genitalia.

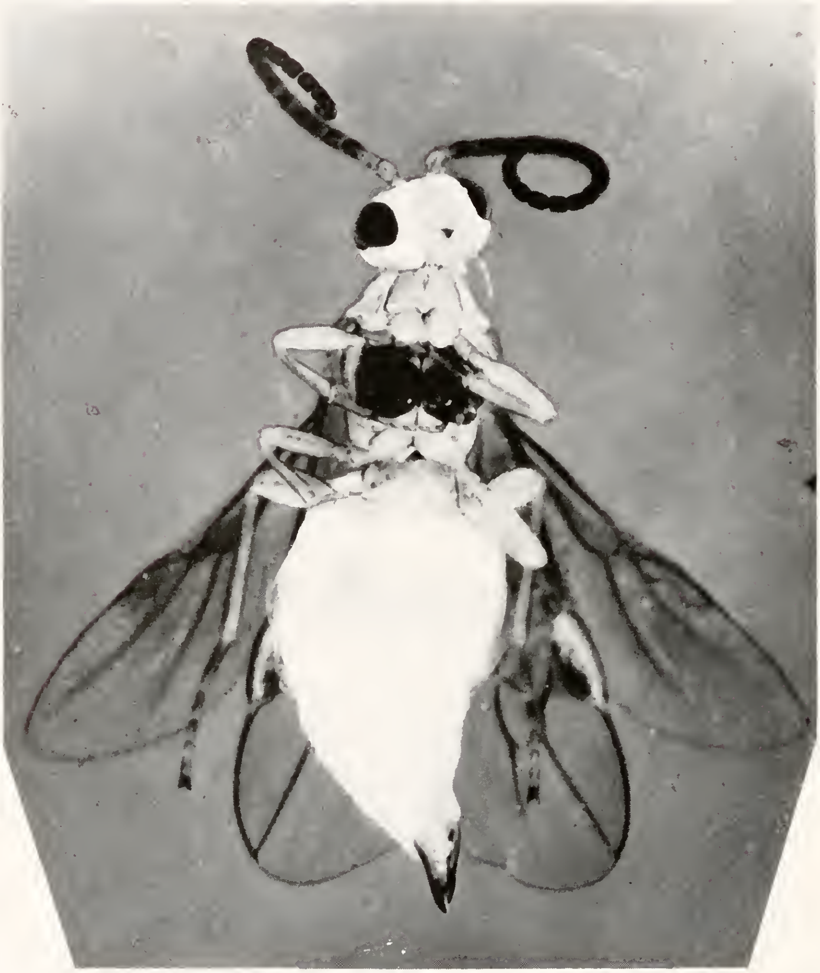

FIGURE 1

In Habrobracon, normal haploid males have cells almost as large as the corresponding cells of diploid females and in some stocks they are actually larger (**Grosch,** 1945). Cells of diploid males are much larger than are those of females or of haploid males. These relationships have been determined by counts of microchaetae within a given area on the upper surface of the wings, each microchaeta corresponding to a single cell. Study of the dispersion of microchaetae in freak 994 showed its cell size to be within the range for the female or haploid male and therefore much smaller than that characteristic of the diploid male. The marked shift of the intersex in the male direction does not then affect the size of its cells. It may be fundamentally female, heterozygous for the sex factor. This condition perhaps prevents the abnormal expansion of cell size while permitting development of antennae and ocelli of normal male type.

The nine intersexual females previously reported had internal abdominal structures as in the female with normal poison sac and glands and seminal receptacle. Each ovary, however, appeared to be a pair of sacs of oogonia showing no differentiation of nurse cells and ova. Serial sections were made of the abdomen of freak 994 and the internal structures were studied. The digestive tract is entirely normal with the crop greatly distended from honey water feeding. A poison apparatus is present but imperfectly developed and situated near the median plane, directly dorsal to the compound posterior nerve ganglion instead of being shifted laterad to the digestive tract. The poison glands are normal although of somewhat small size. Their ducts converge to a common duct connecting distally with an imperfect poison "sac" and proximally traversing the very short distance to the region where normally lies the root of the sting. The poison "sac," of approximately normal length, is reduced in diameter to an irregularly sclerotized strand. It is surrounded by longitudinal muscles as in a normal female. Nothing corresponding to a seminal receptacle could be located, nor were any gonads to be found. The fat body appears normal, surrounding the digestive tract and the poison apparatus dorsally and laterally.

DISCUSSION

In the report on the nine intersexual females, it was suggested that they might be accounted for by a dominant mutation in a sex allele changing xb to xb^m. The intersexes would then be modified females, $xa\ xb^m$. A similar hypothesis would cover freak 994, but here the mutation may have been x-ray induced and more potent than in the previous case so that the intersexuality would be more extreme with turning-point earlier in development.

Failure to find gonads in freak 994 does not necessarily mean that they were lacking from the beginning for they may have begun development and then disintegrated.

Comparison may be made between freak 994 and certain types of "deficient" individuals previously reported in Habrobracon (Whiting, 1926). Some of the "deficient" had external genitalia lacking but gonads present. Others had testes of reduced size, or present on one side, lacking on the other. Some of the "deficient" females with no trace of poison apparatus had well differentiated ovaries with eggs and nurse cells. This is just opposite to the condition found in the intersexual female, freak 994. There was no intersexuality among the "deficient."

SUMMARY

An intersexual female developed from a heavily x-rayed egg fertilized by an untreated sperm. The specimen is more strongly intersexual than a group of nine previously reported, for its external female genitalia are much reduced, its poison apparatus defective and its ovaries altogether lacking. Externally, it appears like a diploid male with small female genitalia.

It is suggested that the x-radiation may have caused a change within a sex-differentiating allele, so that the heterozygote would develop into an intersex rather than a normal female.

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LOCI OF ACTION OF DDT IN THE COCKROACH (PERIPLANETA AMERICANA)

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In the cockroach, DDT produces symptoms which clearly reflect involvement of the neuromuscular apparatus. These are qualitatively much the same in all arthropods which have been studied, though there are important quantitative differences. Thus, in any given animal the time course of the poisoning is a function of dose, and for a dose of comparable toxicity in terms of final mortality, the symptoms unfold and death occurs much more rapidly in some insects (the fly) than in others (the roach) (Tobias, Kollros, and Savit, 1946a). In the roach, the sequence of symptoms is initiated by hyperextension of the legs, elevation of the center of gravity and development of postural instability. The hyperextension then decreases and is superseded by increasing and generalized tremulousness, involving the head, body, and all appendages; the gait becomes ataxic, and minor stimuli of sound or touch result in great hyperactivity, exhibited mainly in running and climbing. The animal falls on its back time after time until finally it can no longer right itself. Leg movements continue in the supine insect with two components, a high frequency intermittent tremulousness and a slower incoordinated flexion and extension. These two types of activity possibly reflect the double innervation which has been described for cockroach muscle (Pringle, 1939), one fiber type producing relatively slow tonic contractions, the other producing relatively fast twitches. It will be seen later that after poisoning these two types of movement can be independently altered. Activity finally diminishes progressively. The fast tremors disappear first and finally there remain only occasional isolated movements of body wall, tarsi, palpi, cerci, or antennae. When no further somatic movement can be detected, the heart usually continues to beat for some time, and electrical stimulation of the nerve cord may still evoke muscle responses. The animal may live in this condition for a day or so and finally die.

Mammals exhibit a similar symptomatology up to a point. In the rat and dog, given DDT intravenously or orally, muscular fibrillations and excessive blinking are followed by tremulousness, ataxia, falling and gross convulsive seizures. The animal may have a number of convulsions and die in the tonic phase of one or recover after gradual subsidence of symptoms. There is no period of prostration and nearly complete immobility as in the insect, because death occurs when systematic respiratory movements cease. In the insect, the small amount of body movement and twitching sufficiently augment diffusion for respiratory exchange. Then too, the insect is far more resistant to anoxia than is the mammal (Wigglesworth, 1939).

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[†] This work was carried out under contract with the Medical Division of the Chemical Warfare Service.

The frog, as might be expected, responds more like the insect than the mammal (Tobias, Kollros, and Savit, 1946b). Respiratory exchange through the skin can sustain life, and, after a period of hyperirritability, the animal lies prostrate and more or less immobile. Such symptomatology has prompted a number of investigations designed to discover a locus of action of DDT. As will be seen, there probably are a number of sites of action depending largely on dosage, but this point of view was only gradually attained.

In mammals (Crescitelli and Gilman, 1946), DDT apparently does not act directly on either muscle, myoneural junction or spinal cord. Since tremors persist after decerebration and mesencephalic transection, and since abnormal cerebral and cerebellar electrical activity persists after atlanto-occipital transection, neither cerebral cortex nor basal ganglia can be a critical site of action, and intact spinal afferents are obviously not necessary for the central effect. The cerebellum is considered, by these authors to be the most likely critical site of action in the mammal. Locus of action has also been investigated in insects. In Drosophila (Bodenstein, 1946), DDT seems not to act on muscle or myoneural junction, but does act on peripheral nerve and may act on the central nervous system. In the cockroach (*Periplaneta americana*), DDT has been found to act on nerve in high concentrations (Yeager and Munson, 1945), and, in low concentrations, on peripheral receptors (probably proprioceptors) (Roeder and Weiant, 1946). The latter workers also have evidence which they interpret to mean that high concentrations may act directly on either the myoneural junction or muscle itself. In the crab (*Cancer irroratus*) there is evidence for action on motor nerves (Welsh, 1946).

It was the purpose of this study to further investigate loci of action of DDT in an insect. Because of its large size and ready availability, the cockroach (*Periplaneta americana*) was used throughout.

METHODS

Cockroaches were immobilized by exposure to 100 per cent CO_2 for 20–60 seconds or by etherization. After CO_2, anesthesia seldom lasted over a minute. Once anesthetized, the roach was fastened to a bit of cardboard by pins passed through either side of the pronotum. Appendages were held in any desired position by pins crossed over the body.

Decapitation was easily achieved by simply cutting the neck with a small scissors. The exposed stump was sealed with low melting-point paraffin. Ligation of the neck prior to decapitation to prevent loss of hemolymph did not prolong survival time. Such animals live about 60 hours (Table I).

To expose a thoracic ganglion, the spinasternum just caudal to the ganglion was cut through, and the incision extended along the sides of the sternal plate. After the plate was reflected forward, removal of superficial fatty tissue and tracheal tubes fully exposed the ganglion. The connectives anterior to the ganglion were held in a jeweler's forceps and cut with iridectomy scissors. Traction on the connectives exposed the lateral nerves, which were sectioned. Finally, the posterior connectives were cut and the ganglion removed. Simple isolation of a ganglion from the rest of the nerve cord can be achieved without excising it by cutting the connectives through slits in the cuticle. Complete transection of the entire roach between sets

TABLE I

Effect of lesions of the central nervous system on symptoms of DDT poisoning in the cockroach

Operation		No. roaches			Average survival, hours	General results	Legs which showed DDT effects
		Controls, no DDT	Operated				
Nature	Site (See Fig. 1)		Before DDT	After DDT		Hyperactivity, tremors and convulsions	
Decapitation	At A	14	—	—	61	Rare tremors in 3 animals—no convulsive activity	None
		—	14	—	56	Typical DDT effects in all animals	All
		—	—	14	56	Typical DDT effects in all animals	All
Transaction of ventral nerve cord	At C and D (both anterior and posterior to thoracic ganglion no. 2)	16	—	—	104	None in any animals	None
		—	15	—	60	Typical DDT effects in all animals	All in 13 animals; 2nd and 3rd pairs in two animals
Destruction of ganglion	Th. 2 (thoracic ganglion no. 2)	15	—	—	77	None in any animals	Leg 2 paralyzed in all
		—	15	—	71	Typical DDT effects in all animals	1 and 3 in 13 animals. 1, 2 and 3 in two animals

of legs results in an isolated segment containing a ganglion, nerves and the attached legs. Such a preparation, if kept moist, is viable for at least 6 to 8 hours.

Excision of the heart largely prevents circulatory removal of substances applied to structures to elicit a local effect. Longitudinal incisions through the cuticle, on either side of the heart tube along its entire length, isolate a strip whose removal carries the heart with it. The heart may be cauterized with equal ease (Yeager and Munson, 1945).

Methods for the administration of measured doses of DDT to insects have been described elsewhere (Tobias, Kollros, and Savit, 1946a).

RESULTS

Localization experiments with uncontrolled DDT doses

Except where otherwise specified, contact poisoning was carried out by confining the roach for 5–15 minutes within a glass cylinder coated with DDT previously precipitated from acetone solution.

Roaches decapitated before or after such contact with DDT behaved like intact poisoned animals (Table I). Therefore, neither the supra- nor the sub-oesophageal ganglia are essential for the development or maintenance of DDT-induced motor activity in the legs or body. Ventral nerve cord connectives were transected both anterior and posterior to the mesothoracic ganglion (Fig. 1, levels C and D). Animals so prepared but given no DDT showed incoordination of the mesothoracic legs when walking, but there were no symptoms which could be confused with those of DDT poisoning. When such animals were subsequently poisoned, however, the mesothoracic as well as the other legs exhibited typical abnormal activity (Table I). After complete transection of the whole body of the poisoned roach, at both these levels (excised segment Fig. 1), leg tremulousness and hyperactivity continued unabated in the isolated segment. The application of DDT emulsion or DDT in acetone to the cut surface of such segments obtained from normal roaches evoked typical DDT effects in the attached legs within a few minutes. The same was true of DDT applied directly to the exposed ganglion in the otherwise intact animal. Emulsion or acetone without DDT had no such effect.

The cells of origin of the leg nerves lie within the lateral halves of the thoracic ganglia, each ganglion in the adult being formed by the midline fusion of two embryonic ganglion masses. Median sagittal section of the ganglion in a poisoned roach (Fig. 1, level F) did not stop hyperactivity in either of the legs innervated from the resulting ganglion halves. Therefore, even half a segment contains all the structures necessary for the maintenance of DDT symptoms in a leg.

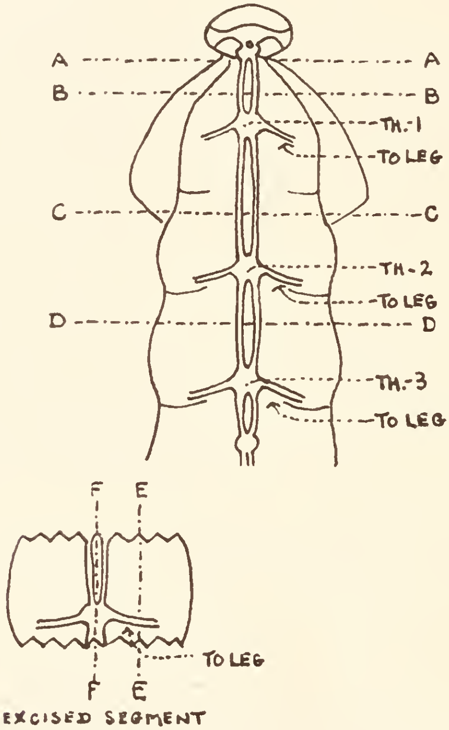

FIGURE 1. Levels of section in cockroach nervous system.

If, however, the entire ganglion was removed the results were generally quite different. Mesothoracic ganglia were removed from thirty normal roaches. The corresponding legs of all were paralyzed and failed to respond to touch or pressure. Shortly after the operation, fifteen of the animals were contact poisoned. All showed typical DDT effects in the pro- and metathoracic legs, but the ganglionectomized mesothoracic legs remained entirely quiet in thirteen and showed only occasional tarsal twitching and some slight movement of the other joints in two. Similarly, ganglionectomy after the development of hyperactivity, rather than before

TABLE II

Experiments on isolated roach segments containing local ganglion, nerves, and legs

No. of segments	Material applied	Route	Number of segments in which there was persistent DDT leg activity
6	Nothing	—	None
3	Emulsion* without DDT	On cut surface	None
2	Acetone without DDT	Injected	None
7	1 Per cent DDT emulsion*	On ganglion	Occurred in all
4	10 Per cent DDT in acetone	Injected into vicinity of ganglion	Occurred in all
3	DDT powder	On ganglion	Occurred questionably in one

* Emulsion—1 per cent DDT, 10 per cent peanut oil, 1 per cent lecithin and 88 per cent 0.90 per cent NaCl (5).

poisoning, either stopped or markedly reduced symptoms in the corresponding legs (Table IV). As was to be expected from these experiments, section of leg nerves lateral to the ganglion stopped or markedly reduced activity in many (65 per cent) of the legs (Table III).

These experiments tentatively suggested that the ventral cord ganglion was critically involved in the motor action of DDT and might itself be a site of action. Conflicting data, however, were also obtained. It was possible, as also reported by others (Yeager and Munson, 1945; Roeder and Weiant, 1946), to produce motor

TABLE III

Visible effect of DDT on amputated legs
(Dose not controlled)

No. of legs	Source of legs	Treatment after amputation	Results after amputation
30	Normal roaches	Normal controls	No spontaneous movement
58	DDT poisoned roaches tremulous and hyperactive	—	Continued activity in 20. No movement in 38
12	Normal roaches	Emulsion without DDT injected into cut end	No movement in any
22	Normal roaches	Emulsion with 1 per cent DDT applied to cut end	Movement in 1, others all quiet
35	Normal roaches	Emulsion with 1 per cent DDT injected into cut end	Movement in 25, other 10 quiet
9	Normal roaches	Acetone without DDT injected into cut end	Movement in 1, other 8 quiet
13	Normal roaches	Acetone with DDT injected into cut end	Movement in 3, other 10 quiet

activity in a large percentage of amputated legs by the injection of DDT emulsion (1 per cent DDT, 1 per cent lecithin, 10 per cent peanut oil, and 88 per cent 0.9 per cent NaCl solution). It will also be recalled that ganglionectomy failed to entirely quiet the legs in two of fifteen experiments (Table I).

Such conflicting data were difficult to interpret. Ganglionectomy or denervation usually stopped leg activity, but this was not invariably the case, and it was possible to produce activity in the amputated legs by injection of DDT. It was suspected that such results might be resolved in terms of DDT dose. Further experiments were then done with measured doses of DDT.

Localization experiments with controlled doses of DDT

It was immediately found that the effectiveness of ganglionectomy in abolishing motor effects was inversely related to dose (Table IV). That is, as the dose of DDT was increased ganglionectomy stopped movement in progressively fewer cases.

TABLE IV

Effect of ganglionectomy on symptoms after various doses of DDT

No. experiments	DDT*	Results of ganglionectomy	
		Number resulting in complete cessation of activity	Number resulting in a reduction of activity
10	Usual moderate contact dose (5–10 mins. in DDT coated tube)	70%	30%
5	Excessive contact dose (approximately 2 hours in DDT coated tube)	20%	80%
25	5–30 mg. DDT per kg. injected intra-abdominally in emulsion**	68%	32%
12	60–70 mg. DDT per kg. injected intra-abdominally in emulsion**	50%	50%
15	130 mg. DDT per kg. injected intra-abdominally in emulsion**	7%	93%

* LD-50 for DDT injected intra-abdominally in emulsion is 20 mg. per kg. (Tobias, Kollros, and Savit, 1946a).

** Emulsion—1 per cent DDT, 1 per cent lecithin, 10 per cent peanut oil, 88 per cent of 0.9 per cent NaCl.

In all cases, however, even when movements were not entirely stopped they were both qualitatively and quantitatively changed. The high frequency tremulousness was always markedly reduced or entirely abolished and the slower movements were much diminished.

Nicotine, in low concentrations, is known to block synaptic transmission centrally as well as peripherally (Libet and Gerard, 1938; Pringle, 1939) but not axonal transmission. When applied to the cockroach ganglion there is an initial burst of electrical hyperactivity (100–800 impulses per sec.) followed by electrical silence (Pringle, 1939). As would be expected, such application of nicotine to a ganglion also produces great motor hyperactivity in the attached leg which can be abolished by amputating the leg (Yeager and Munson, 1945).

Now then, if nicotine applied to a ganglion in a concentration which did not affect peripheral nerve were to stop DDT symptoms, this would be added evidence for the importance of the ganglionic cell bodies or synapses in the development and maintenance of such symptoms. After poisoned roaches became hyperactive the heart was excised. This did not decrease activity, but served to greatly diminish circulatory transport of solutions applied for local effects. Solutions were then applied as small droplets to the ganglion or a region of leg nerve exposed by cuticle excision.

Dilute nicotine solutions (0.01 per cent in insect Ringer) applied to the leg nerves of the normal or poisoned roach did not paralyze the leg. Typical DDT induced activity could not be stopped in this fashion. This was almost surely not due to failure of nicotine to reach the nerve since spontaneous movement as well as that following electrical stimulation of the ganglion could be stopped by a similar administration of 1 per cent novocaine. When, however, this nicotine solution was applied to a ganglion (in the same normal or poisoned animal in which it was ineffective on peripheral nerve) there was a short-lived burst of great activity in the legs of the segment, followed by complete immobility or markedly decreased activity. Since the nicotine was effective in concentrations which did not block peripheral nerve, it was concluded that it was acting by blocking ganglionic synapses and not by spill-over to the emerging nerve roots. It is clear (Table V) that, as in the case of ganglionectomy, the immobilizing effect of nicotine decreased as the dose of DDT increased, and, as was also true after ganglionectomy, if nicotine did not stop activity it considerably decreased and modified it.

TABLE V

Effect of locally applied nicotine and novocaine on motor symptoms of DDT poisoning after various doses of DDT*

No. experiments	DDT**	Number of experiments in which activity was modified			
		1.0% novocaine	0.01% nicotine		
		Injected into tibia	Injected into tibia	Applied to ganglion	
		Complete inactivity	Complete inactivity or reduced activity	Activity stopped	Activity reduced
4	10–30 mg. per kg. applied to body surface 18 hours before	100%	0%	100%	0%
9	100 mg. per kg. applied to body surface 18 hours before	100%	0%	77%	22%
13	500 mg. per kg. applied to body surface			23%	77%
4	1000 mg. per kg. applied to body surface			0%	100%

* All experiments on cardiectomized roaches to prevent circulatory removal of substances applied for local effect.

** DDT applied to surface in acetone. LD-50 for DDT so applied is 10 mg. per kg. (Tobias, Kollros, and Savit, 1946a).

Discussion

It is clear that DDT can produce motor symptoms by effects peripheral to the ganglion. It is equally clear, however, that the ganglion plays a role in the initiation and maintenance of symptoms and that this role is to some extent dependent upon DDT dose.

Roeder and Weiant (1946) found that, in the cockroach, very low concentrations of DDT can initiate centripetally directed, high frequency (300–400 per sec.), temporally irregular bursts of nerve impulses, presumably excited by action of DDT on the campaniform sensilla (presumptive proprioceptors). There was no evidence of any muscle movement which might have initiated such centrally directed impulses. Welsh (1946) has demonstrated that DDT in very low concentrations can also favor repetitive response of motor fibers (*Cancer irroratus*) to a stimulus normally evoking single responses, and Yeager and Munson (1945) have concluded that high concentrations can produce similar changes in the cockroach.

The results of ganglionectomy, in the cockroach (surgical or nicotine inactivated), after various doses of DDT, support the view that the initiation and continuation of the hypermotor symptoms of DDT poisoning after low doses of DDT require an intact sensori-motor reflex arc, and that random afferent impulses in sensory nerves may indeed, as suggested by Roeder and Weiant, excite motor neurones in the ganglion to initiate incoordinated muscular activity. From the experiments here reported, this would appear to be a part of the common sequence of changes in the roach poisoned by uncontrolled contact doses. The fact that ganglionectomy becomes less and less effective as the dose of DDT is progressively increased would suggest that larger amounts of DDT may act directly on motor nerves. Obviously, these data do not rule out a possible direct action on muscle. Within the dose ranges which have been used there is, however, no conclusive evidence for a direct action on muscle. This is not to say that such action could not occur at some sufficient dosage level. In addition, ganglionectomy is seen to have stopped the rapid tremulousness after any dose which was tried, suggesting that the high frequency movements may be initiated reflexly rather than by direct action on motor fibers at large as well as at low doses of DDT. This general picture is compatible with the subsidence of high frequency tremulousness before subsidence of slower muscular activity. If the former is reflex and the latter due to direct nerve action one might expect this order of dropping out on the basis of much greater fatigability for the reflex arc than for the nerve trunk.

Pattern development of symptoms

It has been claimed (Laüger, Martin, and Müller, 1944) that if DDT be put on one leg of a fly the development and progression of symptoms follow a definite, orderly and reproducible path from leg to leg. Such a phenomenon might be very important indeed for an understanding of the mechanism of DDT action. The authors have not been able to confirm this finding.

Conclusions

1. Neither decapitation, section of one or several nerve cord connectives nor complete transection of the entire insect body at one or several levels between nerve cord ganglia prevents the development of the typical motor effects of DDT in any

of the legs of the cockroach. After combined antero-posterior isolation of a nerve cord ganglion, even median sagittal section of the ganglion does not prevent motor symptoms in the legs still attached to the lateral ganglionic cell masses. Therefore, the anatomical elements necessary for development of the motor symptoms of DDT are contained within the lateral half of a body segment which contains the lateral half of a ganglion, leg nerves and peripheral structures.

2. Since the motor symptoms of DDT poisoning can occur in amputated legs, in legs whose nerves have been cut, and in legs whose segmental ganglia have been destroyed, it is possible for DDT to produce its motor effects by action on some structure or structures peripheral to the segmental ganglion.

3. The motor symptoms of DDT poisoning can be stopped or diminished in a leg by ganglionectomy, leg nerve section, or ganglion synaptic block with nicotine. The effectiveness of these procedures is in inverse relation to the dose of DDT administered. These findings suggest that, in the cockroach, low doses of DDT may excite motor fibers reflexly by impulses fired into the ganglion over afferent nerve fibers, whereas high doses may act on elements on the motor side of the ganglion and thus not require an intact reflex arc. Since ganglionectomy stops the fast component of the hypermotor activity, however, equally well after large or small doses of DDT, this component may be reflexly initiated and maintained after all doses of DDT.

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TILLINA MAGNA: MICRONUCLEAR NUMBER, ENCYSTMENT AND VITALITY IN DIVERSE CLONES; CAPABILITIES OF AMICRONUCLEATE RACES

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It is well established that the number of micronuclei in *Tillina magna* is highly variable. For example, Gregory (1909) found 6–10, and Ilowaisky (1921), in a ciliate which he called *Pseudocolpoda cochlearis cienkowskii,* reported 2–6. An examination of Ilowaisky's text and figures shows conclusively that his ciliate was *T. magna* Gruber (1879) as Kahl (1931, p. 282) pointed out. Kahl apparently regarded six as the typical number. Bresslau (1922) observed the nuclei in sufficient detail to note the extrusion of macronuclear material at division and the presence of several micronuclei, though he reported no counts of their actual number. The writer (1946), in a study dealing chiefly with the history of the nuclei during division and encystment, counted the number of micronuclei in 100 individuals (50 active and 50 encysted) of each of three clones, and found that it varied from 6 to 11 in one clone and from 4 to 6 in the other two. Thus the number was found to vary in different individuals of the same clone, and the mean number was found to vary in different clones. Active specimens and resting cysts of any particular clone had on the average like numbers of micronuclei. Contrary to statements in the literature, it was shown that the micronuclei divide at the time of cell division, and not indiscriminately or without regard to cell division. The mechanism by which two daughter cells may receive unlike numbers of micronuclei at division, thus accounting for variations in number within a clone, was described.

The significance of the wide variation in micronuclear number is unexplained. Structurally and physiologically an individual having only 4 micronuclei does not appear to be fundamentally unlike one having 11 micronuclei. The same condition prevails in the closely related species, *T. canalifera,* which I was formerly disposed to regard (1945) as identical with *T. magna*. However, on the basis of information furnished me by Dr. George W. Kidder, of Amherst College, it appears that *T. canalifera* merits recognition as a valid species, chiefly because of the very conspicuous nature of its canal system. In *T. canalifera,* Turner (1937) reported 4–14 micronuclei, though Burt, Kidder, and Claff (1941), in specimens obtained from the late Dr. Turner, found only one. Hence, it is clear that the micronuclear number may vary from 1 to 14, yet the evidence indicates that the uninucleate and multinucleate races were equally cultivable, vigorous, and capable of producing normal resting cysts.

The present study of *T. magna* was undertaken in order to obtain additional information concerning two points: (1) the normal variation in micronuclear number in various natural races and (2) the significance of such variation. The investigation of the first point is readily feasible, in that the micronuclei may be counted with absolute certainty in Feulgen preparations of favorably oriented resting cysts or medium-sized trophic specimens. The investigation of the second point, though less suited to direct approach, is not impracticable. A number of questions arise, some of which submit to experimental analysis. For example, is the number of micronuclei related in any way to size, whether of trophic specimens, division cysts or resting cysts; to division rate; to vitality, meaning capacity to endure with undiminished vigor as generations pass; to ability to produce resting cysts; to the viability of such cysts; or to ability to excyst? Of the foregoing measurable characters, the following were selected as being most readily amenable to experimental investigation: ability to produce resting cysts, size and viability of such cysts, capacity to excyst, division rate and vitality. These then, will be considered in relation to micronuclear number, though not all of them will receive equal consideration. The study assumed unlooked-for interest when it became evident that three of the races were amicronucleate. Thus a comparison of the potentialities of micronucleate and amicronucleate clones became possibile.

Materials and Methods

Twenty clones of *T. magna,* to be designated numerically, were used in the study. The progenitors of these respective clones were collected in a meadow, known locally as Sparrow's Pasture, in the vicinity of Chapel Hill, North Carolina. Comparisons with clones from other sources were desirable, but unfortunately attempts to collect *Tillina* elsewhere in the Chapel Hill region, and in the vicinity of Stanford University, California, and Woods Hole, Massachusetts, were unavailing. In this study a clone refers to all the progeny which were derived asexually from a single resting cyst or trophic specimen. The intervention of encystment and subsequent excystment is not considered to be a valid reason for changing the clonal designation, since there is no evidence that encystment in *Tillina* involves a sexual process which might change the genetic constitution of the clone. (It should perhaps be recalled that *Tillina,* like its near relative *Colpoda,* reproduces within a thin-walled temporary cyst, from which usually four progeny emerge shortly as a result of two successive divisions. The term encystment, as used in this study, does not refer to these temporary division cysts, but to the protective or resting cysts.) It is not definitely established that all of the twenty clones were genetically different, since their histories prior to their period of laboratory life were unknown.

The progenitors of clones 1, 2, 6, 8, 9, 11, 12, 13, 15, 17, 18, and 19 were taken as active specimens on Sept. 10, 1945, and these clones were therefore cultured simultaneously in the early part of the study. Eight of the foregoing clones, namely, 6, 11, 13, 18, 15, 19, 1, and 17, have already been reported on briefly under the numerical designations 1 to 8, respectively (Beers, 1946a). In the present paper my original numerical designations of all clones have been changed for the convenience of the reader in using the accompanying tables. The progenitors of clones 3, 14, 16, and 20 were isolated on Feb. 4, 1946, when dried leaves and debris, after 8 months of storage at 19° C., were immersed in weak hay infusion; these clones were therefore cultured simultaneously. It is evident that they were derived from dried cysts. The progenitors of clones 4, 5, 7, and 10 were isolated on April 8, 1946, when moist leaves and debris, which had recently washed against the bases of willow saplings in the meadow, were immersed in hay infusion; these clones were maintained in culture simultaneously toward the end of the study. They were undoubtedly derived from wet cysts.

An attempt was made to maintain each of the clones in pure-line culture for a period of 60 days. Sixteen of the clones were readily cultivable and continued with undiminished vigor throughout the period; four were intractable in that their division rates declined and the lines encysted well before the end of the period. Thus the laboratory histories of the clones varied, although the conditions of culture were uniform. The details, in relation to micronuclear number, will follow.

Each clone was cultured in depression slides in the form of four sub-lines. These were maintained at 23° C. in 0.05 per cent lettuce infusion to which suitable quantities of *Pseudomonas fluorescens,* grown on nutrient agar, were added as food. Previous experience has shown that this general procedure, combined with daily isolations and transfers to fresh environments, meets adequately the cultural needs of *Tillina* (Beers, 1944, 1945). Records were made daily of fission rates and other points of interest.

Surplus animals from the lines were stained on cover glasses by the Feulgen method in order to make micronuclear counts of active specimens. Small stock cultures of each clone furnished precystic specimens when the food supply neared depletion. These specimens were removed and allowed to encyst on cover glasses in the manner described by Beers (1946). Thus convenient preparations were available, first for making measurements of living cysts, and then for Feulgen staining. All measurements and micronuclear counts of cysts were made on *single* resting cysts. These are the common type. They are practically spherical and therefore well suited for making accurate measurements.

NORMAL VARIATION IN NUMBER OF MICRONUCLEI

The data bearing on diversity in micronuclear number in the twenty clones are summarized in Table I, in which the clones are arranged and numbered in the order of decreasing mean numbers of micronuclei. The data, ignoring for the present the mean diameters of resting cysts, are largely self-explanatory. A few points deserve special mention.

In any particular clone both active specimens and resting cysts showed practically the same extremes of variation (range) in micronuclear number and had essentially the same mean number of micronuclei.

In different clones the mean numbers of micronuclei were extremely variable. Some clones (e.g., 1 and 2) had consistently high mean numbers; others (e.g., 16 and 17), consistently low numbers, with many intergrades between these extremes.

Clones 18, 19, and 20 were amicronucleate. This statement is not based on casual observation, but on an intensive study of these clones. In trophic specimens and resting cysts the micronuclei of *Tillina* are not disposed toward secretiveness. They are never imbedded in the macronucleus. Each has an endosome which stains intensely and conspicuously by the Feulgen method. In mature resting cysts only the rod-shaped or ellipsoid macronucleus and the micronuclei stain to any appreciable extent; there is nothing in the cytoplasm to conceal the micronuclei. In trophic specimens it is true that the food vacuoles also stain, but the micronuclei always lie in the clear peri-macronuclear space and are not in a position to be concealed by the vacuoles. Moreover, considerable numbers of individuals of clones

18, 19, and 20 were stained. These included not only the usual resting cysts and medium-sized trophic specimens, but also young cysts, cysts in the process of excystment, and individuals just excysted. None showed a micronucleus, whereas individuals of the remaining seventeen clones, stained at the same time by the same method, invariably showed micronuclei.

The individuals of some clones (e.g., 1, 3, 5, 12) showed great diversity in micronuclear number within the clone. This fact is brought out clearly by the range which is cited for these clones, and it is further emphasized by the high standard deviations in the clones. Clone 12 showed the greatest degree of heterogeneity in that the range in micronuclear number extended from 2 to 11, with all intervening numbers being represented. On the other hand, some clones (e.g., 2, 4, 8; 13, 15, 16, 17) were relatively homogeneous, with narrow ranges and low standard deviations. Other clones lay between these extremes. Only the amicronucleate clones showed complete homogeneity.

Thus, it is seen that individuals of a clone exhibit varying numbers of micronuclei, that clones differ with respect to their mean number, and that amicronucleate clones exist in nature.

The mean number of micronuclei in the 850 micronucleate active specimens of Table I (representing 17 clones) was 7.06; the mean number in the 850 micronucleate cysts was 7.08. Unfortunately, the number in the progenitor of each clone

TABLE I

Tillina magna. *Variation in number of micronuclei in twenty clones; relation of micronuclear number to size of cysts. The clones are numbered and arranged as the mean number of micronuclei (average of means for fifty active specimens and fifty resting cysts) decreases.*

Numerical designation of clone	Range in number of micronuclei		Mean number of micronuclei ± standard deviation		Mean diameter of 50 resting cysts in microns ± standard deviation
	50 active specimens	50 resting cysts	50 active specimens	50 resting cysts	
1	10–16	9–16	12.90±1.87	12.32±1.93	85.76± 9.65
2	8–12	9–12	10.48±0.94	10.96±0.87	82.94± 7.65
3	7–14	6–13	9.62±2.30	9.24±2.08	79.68± 8.72
4	7–10	7–10	8.52±0.82	8.60±0.95	88.62± 7.25
5	6–14	6–12	8.17±1.95	8.28±1.66	88.36± 9.61
6	6–10	6–10	7.56±1.28	7.78±1.24	93.60± 6.21
7	5–10	5–10	7.25±1.28	7.38±1.36	92.44± 7.08
8	6– 9	6– 9	7.16±0.85	7.06±0.92	78.50± 7.82
9	5– 9	4– 9	6.52±1.15	6.74±1.21	84.16± 8.62
10	4– 8	4– 8	5.98±1.52	5.94±1.46	85.42± 5.28
11	5– 8	5– 9	5.90±1.03	5.76±0.96	86.14± 5.34
12	2–10	2–11	5.74±2.41	5.90±1.97	85.64± 5.32
13	4– 6	4– 6	5.12±0.42	5.10±0.45	80.64± 8.92
14	4– 8	4– 8	5.12±1.51	4.98±1.42	84.18± 4.76
15	4– 6	4– 6	4.86±0.53	5.20±0.57	81.60± 8.41
16	4– 6	4– 6	4.92±0.63	4.98±0.75	80.92± 7.37
17	3– 5	3– 5	4.28±0.75	4.14±0.74	91.42± 6.87
18	—	—	0	0	86.28± 5.63
19	—	—	0	0	84.32±10.36
20	—	—	0	0	81.52± 5.34

is unknown, since the micronuclei cannot be identified in living specimens. It seems reasonable to assume that the progenitor of each had a number approximately equivalent to the mean which was determined for the clone, and that it produced some offspring having fewer, and some having more, than its own number.

It is well known that the number of micronuclei is variable in many species of ciliates. Thus, *Paramecium multimicronucleatum* has 2 to 7 (Powers and Mitchell, 1910), though usually 4 (Wenrich, 1928); *Spathidium spathula*, 6 to 9 (Maupas, 1888, p. 247); *Urostyla grandis,* 10 to more than 40 (Tittler, 1935); and *Stentor coeruleus,* 10 to 42 within a single clone (Schwartz, 1935). On the whole, such variations within a species appear to have little effect on the structure or behavior of the individuals and to be without functional significance. This general conclusion is supported by the observations on *T. magna* which follow immediately.

Number of Micronuclei in Relation to Various Aspects of Cystment

All the clones produced normal resting cysts upon the depletion of the food supply in small stock cultures prepared with surplus animals from the lines. Furthermore, all the specimens in such cultures encysted; none persisted in prolonged swimming, thereby to perish of starvation. Hence, it is clear that the ability to encyst is not dependent on the presence of the micronucleus, since amicronucleate as well as micronucleate clones were able to encyst. Moreover, the cysts of all the clones remained viable for many months. They could be activated at any time after the fourth day by immersion in distilled water or 0.05 per cent lettuce infusion. From 2 to 2.5 hours were required for emergence at 23° C., and practically 100 per cent of the specimens excysted. No precise figures are given here, since the percentage of excystment under various conditions has been dealt with in a previous paper (Beers, 1945) and the present study contributes nothing new on this point. However, the present results show clearly that the viability of resting cysts and their capacity to excyst are in no wise related to the presence of a micronucleus, or to the number of micronuclei. Well over 90 per cent of the cysts produced in the various clones were single ones; double cysts appeared sporadically, some in amicronucleate clones, some in micronucleate. Amicronucleate cysts undergo the usual colpodid type of macronuclear reorganization, involving the extrusion of a portion of the macronuclear substance into the cytoplasm (Taylor and Garnjobst, 1941; Burt, Kidder and Claff, 1941; Beers, 1946).

The size of the cysts in different clones was made the subject of special study, for it was thought that the number of micronuclei might affect the size of the cysts. The diameters of fifty living single cysts of each clone were measured, each measurement extending from the outer surface of the ectocyst of one side to the corresponding surface of the other. The results of these measurements are included in Table I. An inspection of the table shows at once that the calculation of coefficients of correlation between micronuclear number and cyst size would be of little value, since cyst size is independent of micronuclear number. For example, if we consider certain extremes in micronuclear number, it is seen that the cysts of clone 1 had a mean diameter of about 85 μ, and those of clone 19 a diameter of 84 μ, with approximately equivalent standard deviations. Clones 2 and 20 and clones 4 and 18 constitute other examples of extreme disparity in micronuclear number with close agreement in cyst size. Among the micronucleate clones, other examples of wide divergence in micronuclear number, yet with general uniformity in cyst size, are furnished by clones 3 and 16 and by clones 6 and 17. On the other hand, some clones having widely divergent micronuclear numbers produced cysts of dissimilar sizes, e.g., clones 3 and 17 and clones 6 and 13. Thus it is seen that clones having widely different micronuclear numbers may produce cysts of equivalent mean sizes or of dissimilar mean sizes. It must be concluded that there is no relation between the number of micronuclei and the size of the cysts.

The same conclusion is reached if we adopt another approach and consider cyst size in clones which had similar, or only slightly different, micronuclear numbers. For example, clones 6 and 7 had similar mean numbers of micronuclei and they produced cysts of equivalent mean sizes; clones 15 and 16 constitute a second example. On the other hand, clones 7 and 8 had similar micronuclear numbers, but they produced cysts which differed significantly in mean size; clones 16 and 17 furnish another example. Hence, clones having similar mean micronuclear numbers may produce cysts of equivalent mean sizes or of different mean sizes. Again, it is evident that there is no relation between number of micronuclei and size of cysts. The mean diameter of the 850 micronucleate cysts of Table I was 85.23 μ; that of the 150 amicronucleate cysts was 84.04 μ.

A word concerning the size of individual cysts may be of interest. In any particular clone of *T. magna,* whether micronucleate or amicronucleate, there is usually wide variation in cyst size, even though the cysts under immediate consideration are all produced in the same small stock culture—meaning a Columbia culture dish containing 1 cc. of fluid. Cysts in such a culture often vary in size from 75 μ to 95 μ; extremes of 64 μ and 104 μ have been noted. The factors which affect cyst size appear to be of a complex physiological nature and are therefore not readily identifiable.

The point of greatest interest in this consideration of various aspects of cystment is the fact that amicronucleate and micronucleate clones behaved alike; clearly the micronucleus of *T. magna* plays a negligible role, if any, with reference to encystment, viability of cysts, excystment, macronuclear reorganization within the cysts, and cyst size.

NUMBER OF MICRONUCLEI IN RELATION TO DIVISION RATE AND VITALITY

The cultural histories of the twenty clones are presented in Table II. Clones 1, 2, 3, and 5 could not be maintained in culture for the arbitrary period of 60 days. The remaining clones were maintained with undiminished vigor and were discontinued at the end of the period.

First, the division rates of the sixteen vigorous clones will be considered in relation to micronuclear number. Clones 4, 6, 7, and 8, as has been seen, had relatively high micronuclear numbers (means, about 7 to 8.5). The total average number of generations produced by the four sub-lines of these respective clones varied from 149 to 174. Thus the clones themselves varied with respect to mean division rate. Clones 9–12 had intermediate micronuclear numbers (means, 6 to 6.5); these clones produced from 149 to 167 generations. Clones 13–17 had low micronuclear numbers (means, 4 to 5); they produced from 160 to 176 generations. Thus clones having intermediate or low micronuclear numbers produced in general as many generations, and had therefore the same division rates, as clones having relatively high micronuclear numbers.

Amicronucleate clones 18–20 produced from 154 to 172 generations. Thus the amicronucleate clones had approximately the same division rates as the micronucleate clones; e.g., clone 19 produced almost as many generations as clone 4; clone 18 produced more than clone 10; clone 20 produced about as many as clone 9 or 13. Many kinds of comparisons are possible, and the reader may choose to make other comparisons between amicronucleate and micronucleate clones. For example, clones 18–20 had higher division rates (i.e., produced more generations) than clones 8, 10, and 14; but clones 18–20 had lower division rates than clones 4, 7, and 16. Thus the general conclusion that amicronucleate clones have the same division rates as micronucleate clones is not invalidated. Sections of amicronucleate division cysts showed that the macronucleus undergoes the usual reorganization after each of its divisions, as in normal micronucleate cysts (Burt, Kidder and Claff, 1941; Beers, 1946).

Next, the vitality of the sixteen vigorous clones must be considered. An examination of the number of divisions produced in the successive 5-day periods in any particular clone shows that the clone was dividing as rapidly at the end of the experiment as at the beginning. Within the time limits of the experiment, the clones showed no decrease in vitality as measured by the division rate. How long the sixteen clones would have continued without diminution in vitality is a question that cannot be answered on the basis of the available data. The important findings are these: Some clones which have relatively high micronuclear numbers are as vigorous as those which have low numbers; amicronucleate clones are fully as vigorous as many micronucleate clones.

Clones 1, 2, 3, and 5 must receive special consideration. As has been said, these clones could not be maintained in culture for the duration of the 60-day experimental period. Clone 1 showed a rapid decrease in fission rate and encysted on the fourth day. Some of the cysts were activated and new lines were established. These in turn declined shortly and encysted. Three additional attempts were made to culture clone 1; each time the lines encysted after 3–5 days. Indeed, clone 1 was so refractory that without these repetitions it would have been impossible to obtain sufficient specimens for the usual number of micronuclear counts. Clones 2, 3, and 5 likewise could not be maintained in culture for 60 days. Their histories are presented in sufficient detail in Table II. Following the encystment of the original lines of these clones, new lines were established with excysted specimens, but they also declined and encysted after 3–4 weeks of culture. It may be maintained that the decline and encystment of these four clones resulted from a failure to meet their cultural needs. However, the conditions of culture were adequate for a total of sixteen clones, and it seems not unreasonable to assume that they were likewise adequate for clones 1, 2, 3, and 5 and to conclude that these clones declined as a result of intrinsic factors.

It has been shown that clones 1, 2, and 3 had higher micronuclear numbers than any of the other clones. Clone 5 likewise had a high number, though slightly lower than clone 4, which was cultivable. The evidence indicates that a large number of micronuclei may be detrimental to the welfare of the organism and incompatible with high vitality, but the number of such clones studied was too small to justify a general conclusion. On the whole, the results show that in *T. magna* the rate of division and the vitality of the race are in no wise related to the number of micro-

TABLE II

Tillina magna. *Relation of number of micronuclei to division rate and vitality in twenty clones maintained in pure-line culture, usually for sixty days. Four clones having high micronuclear numbers encysted before the expiration of sixty days; the remaining sixteen continued with undiminished vigor, though their mean micronuclear numbers (see Table I) varied from 8.56 to 0.*

Successive five-day periods	Total average number of divisions per line for successive periods																			
	Cl. 1	Cl. 2	Cl. 3	Cl. 4	Cl. 5	Cl. 6	Cl. 7	Cl. 8	Cl. 9	Cl. 10	Cl. 11	Cl. 12	Cl. 13	Cl. 14	Cl. 15	Cl. 16	Cl. 17	Cl. 18	Cl. 19	Cl. 20
1	5.5*	13.5	11.5	14.5	15.5	12.5	16.0	11.5	12.5	11.5	13.0	13.0	14.5	14.5	14.5	14.0	12.5	11.5	12.5	13.5
2		11.0	9.5	16.5	12.5	13.0	15.0	12.5	11.5	10.5	14.0	14.5	15.0	13.0	12.5	14.0	11.5	12.5	13.5	12.5
3		9.5	12.5	13.5	11.5	14.5	12.5	12.5	13.0	12.5	12.5	15.5	13.5	12.5	13.0	15.0	12.5	16.5	14.5	11.5
4		7.5*	10.0	14.0	10.0	11.5	14.0	13.0	12.0	15.5	11.5	13.5	14.5	11.5	16.5	15.5	13.5	12.5	15.0	14.5
5			8.5	13.0	7.0	15.0	12.5	11.5	14.5	14.5	14.0	12.5	15.0	14.5	14.0	14.5	14.5	11.0	15.5	12.5
6			9.5	16.5	0.5*	12.5	14.5	11.5	15.0	13.5	13.5	13.5	12.5	12.5	15.5	16.5	13.5	13.0	16.5	14.5
7			7.8	15.0		14.0	15.0	12.5	15.5	12.5	12.5	14.5	14.5	13.5	13.5	14.5	14.0	13.5	14.5	15.5
8			2.2*	13.5		13.5	16.5	13.5	14.0	11.5	14.5	15.0	13.5	14.5	12.5	13.0	15.0	14.5	13.0	14.5
9				14.5		13.0	13.5	11.5	16.0	12.5	13.0	13.5	11.5	11.5	14.5	14.5	14.5	12.5	14.5	13.5
10				14.0		16.0	14.0	12.5	13.5	10.5	15.0	12.5	12.5	14.0	16.0	16.5	13.5	11.0	13.5	12.5
11				15.5		12.5	15.5	13.0	12.5	13.5	12.5	14.5	14.5	12.5	13.5	14.5	13.0	12.5	15.0	13.5
12				14.0		14.0	14.5	13.5	12.0	10.5	12.5	15.0	15.0	13.0	14.5	13.5	12.5	13.0	14.0	15.5
Total number of generations	5.5	41.5	71.5	174.5	57.0	162.0	173.5	149.0	162.0	149.0	158.5	167.5	166.5	157.5	170.5	176.0	160.5	154.0	172.0	164.0

*Cl. 1. Encysted after 4 days.
*Cl. 2. Encysted after 20 days.
*Cl. 3. Encysted after 37 days.
*Cl. 5. Encysted after 26 days.

nuclei, or even to the presence of a micronucleus, since amicronucleate clones proved to be as vigorous as micronucleate clones.

The origin of the amicronucleate races cannot be accounted for with certainty. It is agreed that such races may arise at any of four periods in the life of ciliates generally: at endomixis, by the transformation of all the reconstruction micronuclei into macronuclei; at autogamy or conjugation, by an analogous transformation of all the derivatives of the synkaryon or amphinucleus into macronuclei; or at division, by an unequal distribution of the micronuclei to the daughter cells. By studying dividing individuals of an unusual race of *Paramecium caudatum*, Wichterman (1946) observed the simultaneous production of bimicronucleate and amicronucleate daughters. In *T. magna* conjugation is of rare occurrence, and endomixis and autogamy are unknown. Therefore, it is likely that amicronucleate races usually take their origin in an unequal distribution of the products of division. However, it should not be concluded from the present results that 15 per cent of all *Tillina* clones are amicronucleate; actually such clones appear to be very exceptional. I have stained many specimens during a period of 8 years; all these specimens had micronuclei, except for the members of clones 18–20.

Discussion

The functional significance of the nuclear dimorphism of the Euciliata has long held the attention of protozoologists. In general, the dimorphic condition has been viewed as representing a segregation of idiochromatin and trophochromatin, the former in the micronucleus, the latter in the macronucleus. It was originally assumed, since ciliates normally possess both types of nuclei, that both are necessary for the continued survival of the individual. Although the precise functions of the respective nuclei are difficult to determine, two lines of investigation have supplied pertinent findings, namely, a study of the capabilities of amicronucleate ciliates of natural occurrence and a study of regenerative capacity, survival, and reproduction in ciliates which have been deprived experimentally of either nucleus, or of both, whether by merotomy or other operative procedures.

The existence of naturally occurring amicronucleate races has long been conceded by such authorities as Woodruff (1921), Calkins (1930), and Reichenow (1929, p. 29) and is now accepted as a fact. The potentialities of these races, as revealed by intensive laboratory study, have demonstrated that the micronucleus is not at all necessary for the maintenance of the essential vital processes of the individual, whereas the macronucleus is indispensable. Aside from the absence of a micronucleus and the manifest inability to carry to completion such processes as endomixis, autogamy and conjugation, amicronucleate races of many ciliates do not differ structurally or physiologically from micronucleate ones.

Thus, Dawson (1919; 1920) maintained an amicronucleate race of *Oxytricha hymenostoma* in pure-line culture for 289 generations (4 months) and in small mass cultures for 5 months longer. The absence of micronuclei did not prevent the animals from attempting to conjugate, but these attempts were abortive. Woodruff (1921) cultured amicronucleate races of *Oxytricha fallax* and *Urostyla grandis* for 246 and 128 generations, respectively, and maintained a race of *Paramecium caudatum* in pure-line culture long enough to determine that it was definitely amicronucleate. A few pairs of conjugants occurred in mass cultures of *O. fallax,* but they failed to live when isolated.

Amicronucleate races of other ciliates have likewise been isolated and cultured long enough to demonstrate not only their viability but their sustained vigor and good health. Among these are the following: (1) *Spathidium spathula.* Moody (1912) was unable to find micronuclei in her specimens, though she was able to culture them for 218 generations. It is evident that they were amicronucleate, since the micronuclei of *Spathidium* were observed and counted by Maupas and were found regularly by Woodruff and Spencer (1922). (2) *Didinium nasutum.* Patten (1921) cultured an amicronucleate race, which was derived from an exconjugant of a normal micronucleate race, for 652 generations. Conjugation occurred in the amicronucleate race, but the exconjugants invariably died. The resting cysts were likewise inviable. It is evident that the race arose through the faulty reorganization of the exconjugant. (3) *Paramecium bursaria.* Woodruff (1931) cultured a race characterized by micronuclear instability for 7 years. Neither endomixis nor conjugation was observed. The race was originally bimicronucleate; later it was variable, exhibiting from 1 to 4 micronuclei; then for about 4 years it was unimicronucleate; finally, a derived race showed no micronucleus, although this race was apparently as healthy and vigorous as its bimicronucleate ancestors which were isolated 7 years earlier. Woodruff (p. 543) aptly points out that "whatever function the micronuclear apparatus plays, the somatic life of the animals is not obviously influenced by profound variations in volume or in distribution of micronuclear material." (4) *Urostyla grandis.* Tittler (1935) found amicronucleate individuals in stock cultures which previously contained only micronucleate specimens. They were indistinguishable externally from their micronucleate progenitors, and they flourished in mass cultures for 2 years. Their macronuclear divisions followed the usual complex pattern characteristic of the species. The race produced resting cysts, some of which could be excysted, although endomixis, which usually occurs in the precystic forms, was absent. Evidently some of the cysts were not entirely normal, since they showed a tendency to disintegrate, perhaps because of the omission of endomixis. (5) *Colpoda steini.* Piekarski (1939) studied comparatively the structure and reproduction of a micronucleate and an amicronucleate race and was able to culture the latter for approximately 6 years. Both races were equally cultivable and vigorous. They reproduced within division cysts from which four progeny regularly emerged and they produced normal resting cysts. They showed the same sequence of events in the division of the macronucleus. These events are of special interest, in that eight chromatic (Feulgen-positive) bodies appear in the macronucleus of the young division cyst. Ultimately each of the daughters receives two of them, and thus the behavior of these bodies suggests an equational distribution of chromosomes. Piekarski concludes that the absence of a micronucleus had no recognizable effect on the activities of the animals.

The present study of amicronucleate races of *Tillina magna* likewise demonstrates the adequacy of the macronucleus, not only for long-continued reproduction accompanied by sustained vigor, but also for encystment and excystment. Thus endowed with the ability to produce viable resting cysts, these races would seem to be capable of indefinite survival, even under the changeable conditions of a natural environment.

Still more remarkable, in connection with the capabilities of amicronucleate races, are the observations of Sonneborn (1940) and Kimball (1941) on mating types. In certain races of *Paramecium aurelia* Sonneborn found a small percentage of animals which, upon undergoing autogamy or conjugation, developed a new macronucleus from a fragment of the old macronucleus. Since the micronuclei commonly disappeared in clones produced by these animals and since the mating type never changed at macronuclear reorganization, Sonneborn concludes that "hereditary characters (including mating type) of clones from macronuclear regenerates cannot be directly determined by micronuclei, for they persist in the absence of micronuclei. Mating type must be determined by the macronucleus. . . ." Kimball was able to assign amicronucleate specimens of *Euplotes patella* to definite mating types, since they paired readily with individuals of known mating type. He concludes that "the micronucleus is thus unnecessary for an animal to be of a definite mating type."

With reference to the role of the nuclei in the regeneration of ciliates following merotomy, the results obtained with different species are not always in complete accord. The physical properties of the cytoplasm constitute an experimental variable; a fluid cytoplasm or a rigid pellicle may interfere with the closure of an injury and thus affect regeneration adversely. Balamuth (1940) has presented an excellent review of the extensive literature on this subject. For the present only a few representative examples of regeneration in ciliates will be considered, with special reference to the nuclear components of the merozoa (cell fragments).

It is well known that enucleate fragments of ciliates can neither regenerate nor continue to live, whereas nucleate fragments regenerate successfully and pursue normal lives. These conclusions are particularly evident in Balamuth's six-page tabular summary of the findings on regeneration in the ciliates. As a rule the macronucleus and micronucleus cannot be separated, and a nucleate fragment, as in Dembowska's work (1925) on *Stylonychia mytilus,* usually means one having both macronuclear and micronuclear material.

However, some investigators have succeeded in obtaining nucleate merozoa of the two types desirable for an experimental analysis of the role of the individual nuclei in regeneration; namely, macronucleate (without micronuclei) and micronucleate (without any part of the macronucleus). Reynolds (1932), for example, in work on an amicronucleate *Oxytricha fallax,* found that various types of macronucleate merozoa could regenerate their missing parts and resume their normal physiological activities. Schwartz, using microdissection techniques, was able to remove the entire macronucleus from *Stentor* and yet leave a number of micronuclei in the specimens. These micronucleate individuals never survived. By means of successive operations, he was also able to remove all the micronuclei from a few specimens, leaving a portion of the beaded macronucleus in place. These macronucleate individuals regenerated and could be cultured as pure lines. Thus he produced experimentally an amicronucleate race, which as regards size and division rate was not different from the normal controls. Bishop (1943), employing the ultra-centrifuge as a means of obtaining merozoa of *Oxytricha fallax,* obtained sixty-seven macronucleate fragments, all of which regenerated, and seven micronucleate fragments, none of which regenerated. Twelve of the regenerated macronucleate individuals were cultured as amicronucleate pure lines. Bishop concluded (p. 451) that "the lack of micronuclear material makes no difference in the regenerative capacity, division rate, motility or morphology of *Oxytricha fallax.*"

On the other hand, there is evidence that in some forms the micronucleus, as well as the macronucleus, is necesesary for regeneration and survival. Thus Reynolds found that both nuclei are necessary for the regeneration of merozoa of *Euplotes patella*. This observation is in accord with the results of Taylor and Farber (1924), who, by means of a micro-pipette, removed the micronucleus from fifty specimens of *E. patella,* all of which died within a few days. None produced more than four progeny. Hence, these authors conclude that "the micronucleus plays more than a purely germinal role in the life history of *Euplotes patella*." However, the situation in *E. patella* is confused, for some of Kimball's unimicronucleate double animals produced viable amicronucleate individuals at division. Some of them survived as clones, though with a low division rate; one such amicronucleate clone survived for 341 days. The results show, according to Kimball (p. 30), "that the micronucleus is not essential for continued life in at least some clones of *Euplotes patella*, though its absence results in a marked decrease in vigor." In various species of *Uronychia* (Young, 1922), and in *Uroleptus mobilis* (Tittler, 1938), *Spathidium spathula* and *Blepharisma undulans* (Moore, 1924) both types of nuclei appear to be necessary for the complete regeneration, growth and division of merozoa.

Thus the evidence afforded by the long-continued culture of a number of naturally occurring amicronucleate races demonstrates conclusively that the macronucleus alone suffices for the maintenance of the vegetative life of the organism—meaning by vegetative life such diverse activities as locomotion, food capture, digestion, assimilation, growth, excretion, respiration, reproduction, and maintenance of cell proportions and form. On the basis of this evidence the normal role of the micronucleus in vegetative life appears to be one of relative passivity. The evidence from operative procedures shows that in many ciliates the macronucleus alone is adequate for complete regeneration, as well as for subsequent growth and division, whereas in other ciliates the micronucleus also is necessary. There is no authenticated case on record in which the micronucleus alone is adequate for the maintenance of vegetative functions or for regeneration. Balamuth's thorough survey of the literature leads him to make the following comment in his summary: "Of the dual nuclear apparatus, only the macronucleus can be shown to function in the actual regenerative process. The role of the micronucleus in this connection is as yet unclear; apparently it is more important in the viability of some forms than in others." On the whole, the evidence tends only to emphasize the importance of the macronucleus and to attest to the validity of Calkins' comment (1930, p. 161) on this organelle: "Far from being negligible it is on the contrary probably the most important element of the cell in matters of metabolism, reorganization, and continued cell life."

The tendency to underestimate the importance of the macronucleus in the life of the organism results probably from its apparent monotony of structure. Lacking chromosomes, its division is usually unspectacular. Nevertheless, its mode of origin is not an incidental phenomenon in the life of the ciliate, for both macro- and micronucleus almost invariably have a common and simultaneous origin. Usually they develop from the synkaryon of the conjugant, by divisions which appear to be equational. Again, they develop from the synkaryon of autogamy or from the endomictic micronucleus. Thus they inherit equally from a common nucleus of origin, and each receives equivalent chromatin elements, chief among which are presumably the genes. In the definitive micronucleus these elements retain the ability to organize periodically as chromosomes, and thereby to arrest the attention of the observer. Once in the definitive macronucleus, on the contrary, they never again assemble in the form of chromosomes. However, it is possible that they are distributed at macronuclear division by a mechanism which is fully as effective as the mitotic distribution of chromosomes, though less conspicuous. For example, it is not impossible that they are represented in multiplicate in the macronucleus and distributed at random throughout its substance. Thus each daughter at division would be reasonably assured of receiving representatives of every type of chromatin element. The behavior and the potencies of the macronuclear fragments of Sonneborn's unusual specimens of *Paramecium aurelia* indicate a multiplicate representation of the chromatin elements. In these specimens the forty or more macronuclear fragments grew and segregated during subsequent cell divisions, until there was only one in each cell. Thus each fragment was adequate for the regeneration of a complete macronucleus and for the continued life of the organism, even in the absence of micronuclei. Hence, Sonneborn concludes that "the normal macronucleus must contain at least forty complete and discrete genomes." A more precise mechanism for the distribution of the chromatin elements, involving, for example, a differential streaming of genetically equivalent elements toward opposite ends of a polarized macronucleus, may be postulated. Regardless of the type of mechanism involved in the distribution of the chromatin elements of the macronucleus at division, the fact remains that inheritance in an amicronucleate ciliate is no less precise, to judge by the structure and physiological activities of the offspring, than in a micronucleate ciliate. The fact that the behavior of the macronucleus does not conform to the chromosome theory of heredity in *sensu stricto,* in that chromosomes are absent, may mean simply that a different mechanism for the distribution of the genes is involved.

Whether the macronucleus of amicronucleate ciliates may justifiably be regarded as an amphinucleus—one containing idiochromatin as well as trophochromatin, as Woodruff (1921), Moore and others have suggested—seems doubtful in the light of recent investigations. Thus it has been shown by Schwartz and by Bishop that viable amicronucleate races of *Stentor* and *Oxytricha* may be derived by experimental means from normal individuals in which the idiochromatin and trophochromatin were presumably segregated in the two types of nuclei. The macronucleus of these individuals, following removal of the micronuclei, was adequate to maintain all the usual vegetative activities in the derived amicronucleate races.

In conclusion, the evidence shows that the macronucleus is the essential nuclear element in the vegetative life of ciliates. The micronucleus functions largely, if not solely, in the periodic replacement of the macronucleus and in the production of new genetic combinations, some of which undoubtedly render the species better adapted to survival. The nature of the physiological conditions which call for a renewal of the macronucleus is not clear; that such renewal meets an imperative physiological need is shown by the widespread occurrence of the phenomenon in the Euciliata.

SUMMARY

The number of micronuclei was examined in 50 trophic specimens and 50 resting cysts of each of 20 clones of *Tillina magna,* three of which were amicronucleate.

In any particular clone trophic specimens and resting cysts contained approximately equivalent mean numbers of micronuclei. In different micronucleate clones the mean number varied from 4.21 to 12.61. The mean for 1,700 specimens of 17 clones was 7.07.

The number in the individuals of any particular micronucleate clone was variable; some clones showed relatively little variation, e.g., 3 to 5 micronuclei; others, considerable variation, e.g., 2 to 11 micronuclei. The smallest number observed in any micronucleate individual was 2; the largest, 16.

All the clones produced normal resting cysts upon depletion of the food supply (*Pseudomonas fluorescens*). The cysts of different clones were equally viable and capable of excystment. Their size was unaffected by the number of micronuclei. Amicronucleate cysts showed the usual macronuclear reorganization. Hence, neither the number of micronuclei nor the absence of micronuclei affected encystment, viability and size of cysts, excystment or macronuclear reorganization.

An attempt was made to maintain each clone in pure-line culture for 60 days and thereby to examine the division rate and vitality. Four clones were refractory and encysted before 60 days expired. The remaining 16 clones, including the three amicronucleate ones, survived with undiminished vigor and were discontinued. The 13 micronucleate clones produced from 149 to 176 generations during the 60-day period; the three amicronucleate clones produced 154, 164, and 172 generations, respectively. Hence, the 16 surviving clones showed slight differences in their average daily division rates, but neither the divison rate nor the vitality of these clones was correlated with variations in micronuclear number or with the absence of micronuclei. Division cysts of amicronucleate clones showed the usual macronuclear reorganization after each division of the macronucleus. The four refractory clones had high micronuclear numbers.

Since conjugation is rare and endomixis and autogamy are unknown in *Tillina*, it is probable that amicronucleate races arise at division by an unequal distribution of the daughter micronuclei.

Some of the literature on amicronucleate ciliates and on the regeneration of various types of nucleate merozoa is reviewed. The evidence shows that the macronucleus is the indispensable nuclear element in the so-called vegetative life of the organism, whereas the micronucleus during this period appears to be a relatively passive organelle. Its chief function concerns the periodic replacement of the macronucleus and the production of new hereditary combinations. Special attention is directed to the fact that inheritance in an amicronucleate race is no less precise than in a typical micronucleate race, although the division of the macronucleus is amitotic and usually reveals no suggestion of true chromosomes. It is evident that the hereditary mechanism of amicronucleate races, and perhaps of ciliates generally, differs radically from the conventional chromosomal mechanism of metazoa.

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THE EFFECT OF LOW TEMPERATURE AND OF HYPOTONICITY ON THE MORPHOLOGY OF THE CLEAVAGE FURROW IN ARBACIA EGGS[1]

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When *Arbacia punctulata* eggs are exposed to low temperature during the first cleavage, a pronounced stalk develops between the daughter blastomeres. A stalk also develops at room temperature if the eggs are made to divide in hypotonic sea water or in sea water lacking calcium ion. The development of a conspicuous cleavage stalk is not a normal feature of the first cleavage in Arbacia, although it does occur regularly in some cells; for example, when fibroblasts divide. The object of the work reported here was to examine the conditions under which the stalk is formed in Arbacia and to relate these facts to current theories of the mechanism of cleavage. These particular experimental treatments were used because they were found to affect the appearance of the cleavage stalk.

METHODS

Eggs of *Arbacia punctulata* in the first cleavage served as experimental material. Ovulation was induced by the removal of the oral half of the test; eggs emerging from the genital pores were collected in a dish of sea water. The eggs were allowed to settle and the sea water was decanted after which fresh sea water was added. Two such washings were carried out to minimize contamination by coelomic fluid. Fertilization was effected by the use of diluted "dry" sperm, and the sperm were never more than one hour old.

The fertilization membranes were removed by shaking. A heavy suspension of eggs was placed in a five-inch test tube, one-half full of the suspension, and shaken rapidly thirty times. Eggs so treated cleave in time with the controls. The best time for treatment is at 2½ minutes after fertilization, for if shaken earlier, many exovates are formed, and if shaken later, many eggs retain the fertilization membrane. The alternative method of removing the fertilization membrane by treatment with the hatching enzyme (Ishida, 1936) was not attempted.

The hyaline layer was removed in a few experiments by washing the eggs in calcium-free artificial sea water. This was accomplished by several decantations and additions of the calcium-free mixture. It was found that the hyaline layer regenerates somewhat if the eggs are returned to a solution possessing calcium ions; hence if eggs are to lack the hyaline layer, they must be allowed to cleave in the calcium-free mixture.

This study was largely accomplished by photographic means. Photomicrographs taken at intervals with Leica-Ibso apparatus, were projected as negatives (1,000 ×) and measurements made with dividers.

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[1] This investigation was aided by a Grant-in-Aid from the Sigma Xi Alumni Research Fund.

Temperature was maintained by means of a thermostatically controlled, water jacketed, glass well, mounted on the microscope stage and connected through a centrifugal pump to a water bath. By this means temperature could be maintained within ± 0.2° C. at or about 20° and within ± 0.4° C. at or about 10° C.

Artificial sea water lacking calcium ions was compounded according to the method of Shapiro (1941). This solution has an osmotic pressure and pH closely similar to that of normal sea water.

The hypotonic solutions were prepared either by the dilution of normal sea water or of the calcium-free mixture.

A few observations are presented on polyspermic eggs cleaving to three or to four cells in one division. Polyspermic development was induced by the method of Smith and Clowes (1924) which involves fertilization in pH 7.2 sea water and the return of the eggs to the normal pH of 8.4 within two or three minutes.

Results

Morphology of the cleavage furrow

The shape of the deepening furrow is markedly different under different conditions; it is influenced by temperature, concentration of calcium ion, tonicity and by presence or absence of the fertilization membrane.

Temperature

At temperatures between 20° C. and 30° C. there is normally no stalk in cleaving eggs whose fertilization membrane has been removed (free cleavage). The furrow is peaked at the apex (Figs. 1 and 2). At low temperatures, 6° to 12° C., a real stalk is formed during the latter part of the furrowing. This occurs whether the egg is enclosed in the fertilization membrane or not. At these low temperatures eggs undergoing membrane-free cleavage, come to resemble a dumbbell with a handle (Figs. 3 and 4).

Calcium ion or urea

Chambers (1938) described the short cleavage stalk which develops when *Arbacia punctulata* eggs divide in calcium-free solutions at room temperature. He used isotonic mixtures of sodium chloride and potassium chloride. In the present study also a short stalk occurred when the eggs were exposed to calcium-free sea water. Similarly a short stalk was figured by Moore (1930a and b) and by Motomura (1934), after treatment with urea solutions.

Fertilization membrane

It is a common practice to remove the fertilization membrane either by dissolving it in urea solutions or by shaking an egg suspension rather violently. These techniques allow the mitotic axis to become much longer and the furrowing is thus more readily followed. If the eggs are confined in the fertilization membrane at 10° C., the blastomeres tend to stay apart and the walls of the furrow are almost vertical (Figs. 11 and 12). At the end of the cleavage a stalk connects the two blastomeres. If this same experiment is varied so that the eggs divide within their fertilization membranes at 10° C. and in calcium-free sea water, a cleavage stalk likewise develops. In this case, however, the stalk moves eccentrically until it is close to the fertilization membrane (Figs. 30 through 34). The difference is presumably due to the fact that the hyaline layer is dissolved in solutions lacking calcium ion and when the hyaline layer is missing the egg is able to slide around inside the fertilization membrane.

Membrane-free cleavage in polyspermic eggs

Polyspermic eggs may undergo free cleavage to form four cells in the first division. In such cases they frequently divide so that a symmetrical figure is seen from above. In this circumstance the four blastomeres each rest upon the bottom of the glass container (Figs. 15, 16, 17, and 18). Frequently one blastomere rests upon the other three at the end of the cleavage (Fig. 21). The former, more symmetrical type of cleavage is more readily followed. When such an egg begins to cleave it first flattens like a biscuit; at this stage it resembles a balloon around which two rubber bands have been placed at right angles. Such a balloon is flattened on the two surfaces where the rubber bands cross. Perhaps the egg, like the balloon, is subject to greater elastic tension in the region where the incipient furrows cross, and therefore flattens on these surfaces.

As seen from above, the egg periphery is roughly square, with corners rounded (Fig. 15); the wide furrows (at 10° C.) gradually sink towards the center with the apices of the furrows approaching one another. The upper and lower surfaces meanwhile remain relatively flat although the two flat surfaces slowly come together. The whole figure at the stage illustrated in Figure 16 resembles a balloon stretched closely over four tennis balls with two rubber bands placed at right angles. Finally a definitive stalk is formed (Fig. 18).

When the furrows first appear, the egg is to be considered as having two equatorial furrows; that is, two constricting rings (Fig. 29*a*), which cross each other. The quasi-independence of the furrows is demonstrated by some eggs which cleave in a similar way but *in which the furrows incise at different rates* (Figs. 19 and 20). In Figure 29*b* the furrow separating *ab* from *cd* is well in advance of the furrow separating *ad* from *bc*. This type of cleavage leads to a figure like Figure 20.

It appears that this curious type of cleavage is brought about by the development of two new ring-like tensions which develop around the necks of the individual blastomeres after the deeper furrow is well established. As a result of the deep primary furrow, four new isthmuses are established about the necks of the four incipient blastomeres (*cf.*, Fig. 29*b*). Perhaps the most significant feature of

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PLATE I

FIGURES 1 AND 2. Egg cleaving at 20° C. in sea water, fertilization membrane removed.

FIGURES 3 AND 4. Egg cleaving at 10° C. in sea water, fertilization membrane removed.

FIGURES 5 AND 6. Egg cleaving at 20° C. in 65 per cent sea water, fertilization membrane removed.

FIGURE 7. Egg cleaving within the fertilization membrane at 20° C. in sea water.

FIGURE 8. Late cleavage at 20° C. in 65 per cent sea water, fertilization membrane removed.

FIGURES 9 AND 10. Polyspermic egg fertilized in sea water at pH 7.2, transferred to normal sea water at room temperature until cleavage began. Cleaving at 10° C. in sea water.

FIGURES 11 AND 12. Eggs cleaving within fertilization membrane at 10° C.

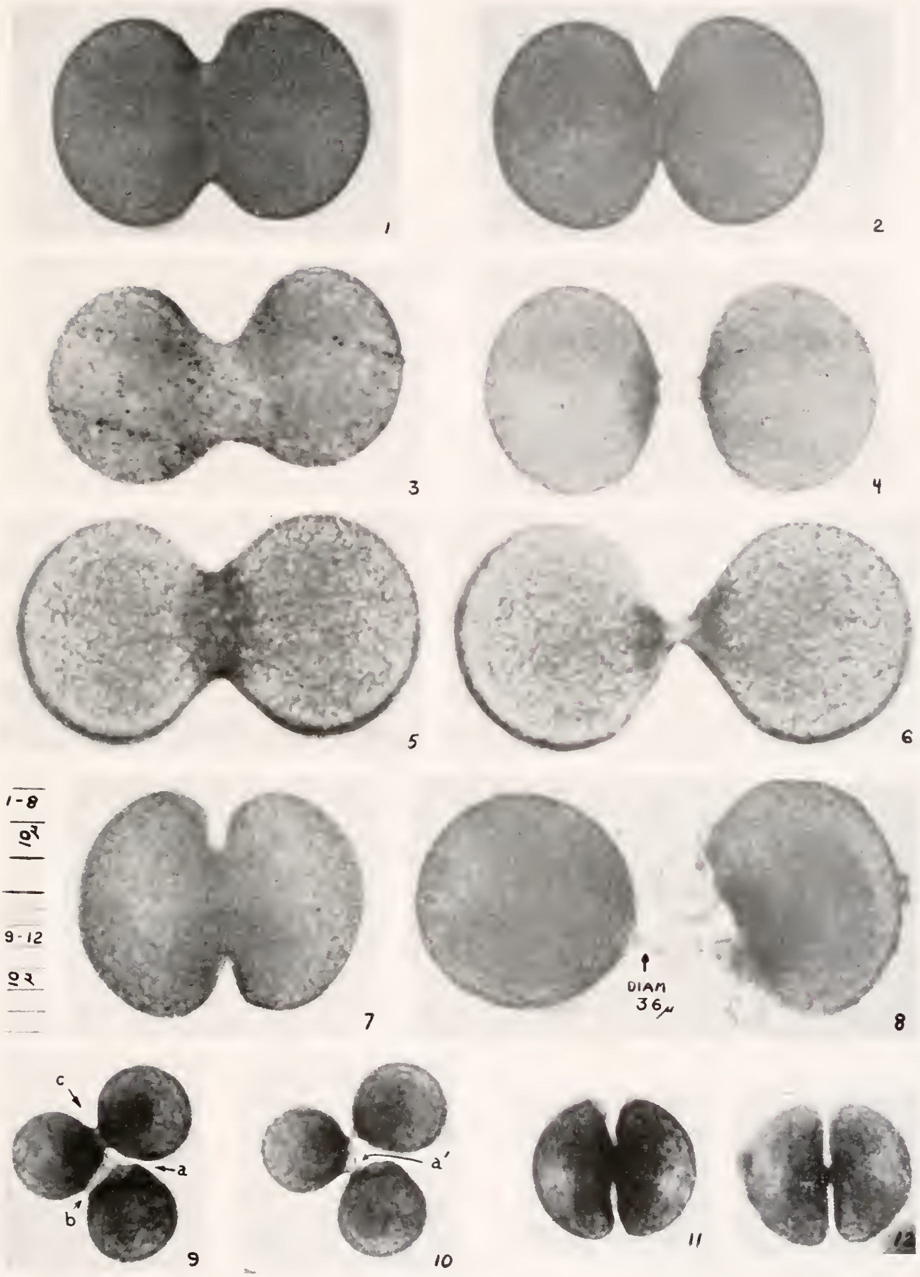
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PLATE I

this type of cleavage is the bridge-like stalk which results (Figs. 23 and 24). In these latter figures note that one circumferential furrow deepened symmetrically and more rapidly than the other. The furrow which started later is very asymmetrical, being much deeper on one side (*cf.*, at the arrow) than the other. Eggs cleaving to three cells show a similar behavior (Fig. 22) and when cleavage is complete they may have a Y-shaped stalk, or if one furrow deepens more rapidly than the others, two stalks may connect to one blastomere (Figs. 9 and 10).

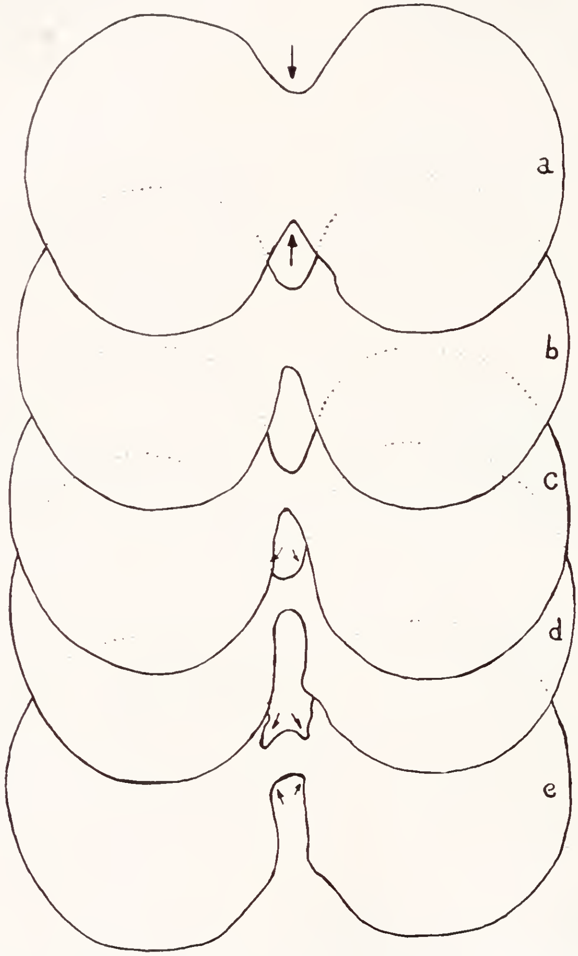

FIGURE 13. Series showing late cleavage and development of the cleavage stalk at 10° C. in sea water.

The speed of furrowing in polyspermic eggs cleaving to four cells may be as rapid as when two cells are being formed, yet it should be remembered that the amount of new surface formed is much greater when a sphere divides into four equal smaller spheres. The surface of a sphere divided into two spheres increases 26 per cent,

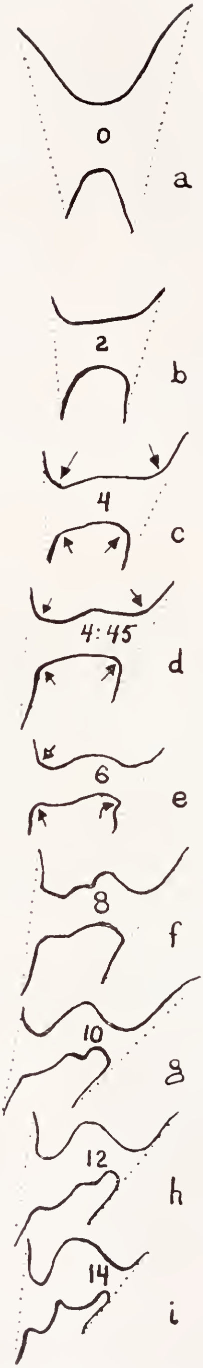
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FIGURE 14. Series showing late cleavage and continued activity of cleavage stalk, during fourteen minutes in calcium-free sea water at 11° C.

if divided to four spheres the surface increases 58 per cent. A polyspermic egg cleaving to four cells forms about 26 per cent more surface than the normal first cleavage but it may do so in the same amount of time.

Membrane-free cleavage in hypotonic sea water and in hypotonic calcium-free sea water

Dilution of the sea water causes a swelling of the egg; it also causes an unusually wide furrow to develop during the cleavage and leads to the formation of a stalk at the end (Figs. 5, 6, and 8). This effect occurs at room temperature (20° C.). The stalk may become very long if the sea water has been diluted sufficiently. In mixtures of 65 parts sea water and 35 parts distilled water, for example, the stalk may finally be 30 micra long. This effect occurs either in the presence or absence of calcium ion. The stalk region is certainly a relatively rigid gel, for it has sufficient rigidity to push the daughter blastomeres far apart. Figure 8 and Figures 40 through 42 show the process of elongation in these extreme cases. Enlarged photographs of the stalk at these stages are shown in Figures 43, 44, and 45 with dimensions noted. In Figure 43 the stalk is only 4.4 micra in diameter at one point. In Figure 44 its minimum width is about 2 micra and it is over 22 micra long. In Figure 45 the constriction is completed. The stalk is still 5 micra wide at some points, but it is less than 3 micra in diameter for a third of its length. Chambers (*ibid.*) relates that a spherical oil drop lying within the egg in the furrow region is not deformed until the "external surface of the advancing furrow is 4 to 5 μ from the surface of the oil." If the egg pictured in Figure 43 has a cortex comparable in thickness, then the stalk must certainly be all gel by the time its diameter is reduced to 7 or 8 μ. One blastomere sometimes ruptures when eggs cleave in 65 per cent sea water. No endoplasm escapes if the stalk has closed. One such closed stalk is shown in Figure 28; it is 5 micra in diameter. The conclusion that the stalk is all gel (and yet it continues to constrict) is a most important conclusion for it strongly supports the contraction theory of cleavage of W. H. Lewis. Close inspection at high magnification fails to show any movement of granules located in the stalk. The active constriction of a 5 μ stalk is recorded in Figures 26 and 27.

PLATE II

FIGURES 15, 16, 17, AND 18. Cleavage of a dispermic egg, cleaving in calcium-free sea water at 10° C. Egg fertilized in pH 7.2 sea water, transferred to sea water at room temperature until beginning of cleavage. Time after fertilization: Figure 15, 72 min.; Figure 16, 74 min.; Figure 17, 88 min.; Figure 18, 190 min.

FIGURES 19 AND 20. Egg showing dispermic cleavage. Treatment as in Figures 15–18. Time after fertilization: Figure 19, 86 min.; Figure 20, 88 min.

FIGURE 21. Dispermic egg. Treatment as in Figures 15–18. One blastomere out of the horizontal plane.

FIGURE 22. Diagram illustrating two types of cleavage to three cells.

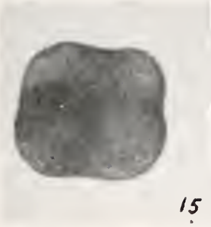
FIGURES 23 AND 24. Egg in 70 per cent sea water at 25° C., after accidental polyspermy. Time after fertilization: Figure 23, 50 min.; Figure 24, 52 min.

FIGURE 25. Dispermic egg cleaving in sea water at 11° C., following fertilization in pH 7.2 sea water.

FIGURES 26 AND 27. Late cleavage of egg in 65 per cent sea water. Room temperature. Time after fertilization: Figure 26, 83 min.; Figure 27, 85 min.

FIGURE 28. Closed stalk following rupture of one blastomere; 65 per cent calcium-free sea water.

FIGURE 29. See text.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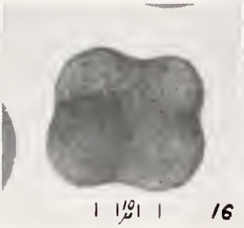

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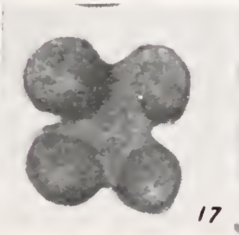
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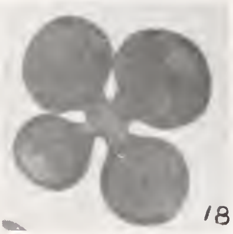
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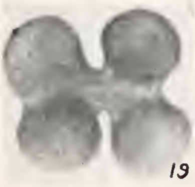
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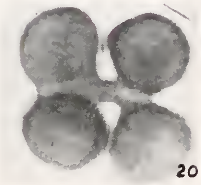
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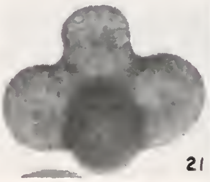
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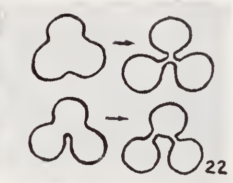
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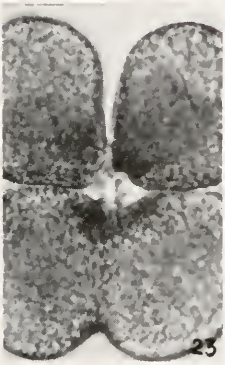
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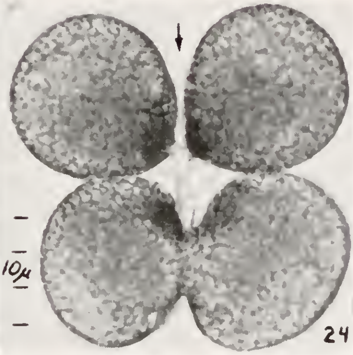

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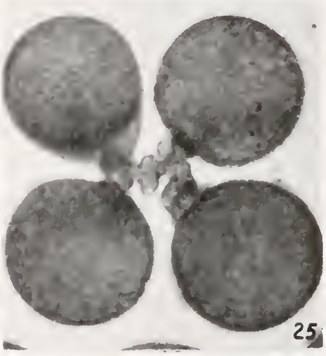
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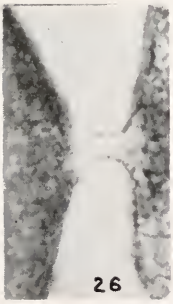
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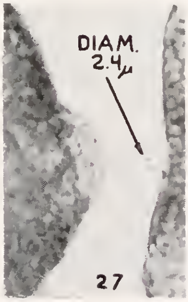

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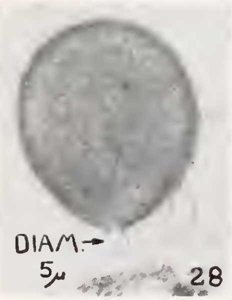

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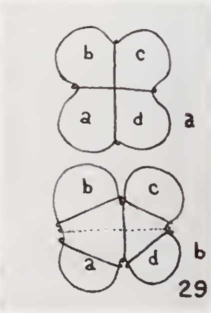
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PLATE II

The stalk

The mitotic axis (greatest length) of eggs undergoing free cleavage becomes progressively longer at 10° than at 20° C. (compare Figs. 1 and 2 with 3 and 4); moreover the early furrow at 10° C. is much more blunt in contour than is the furrow of eggs at higher temperatures. A study of the final phase of cleavage under high power (Fig. 13) shows how the wide furrow is transformed into a stalk.

In Figure 13*a* the deepening furrow is still blunt with a diameter of about 14 micra. In Figure 13*b*, however, the stalk is beginning to square off. The arrows (Figs. 13*d* and *e*) indicate the region where the constriction is most active. The details are similar and are very clear in eggs cleaving in calcium-free sea water at 10° C. The series of diagrams shown in Figure 14, *a* to *i*, again show that the broad furrow first deepens until the diameter of the waist is about 7 or 8 micra (*a* and *b*), then the stalk is elongated by the constriction of the subequatorial cortex (*c* and *d*, see arrows); meanwhile the entire stalk is diminishing in diameter. The minimum diameter of the stalk is about 4 micra at 10° C. and in calcium-free sea water; in hypotonic solutions the diameter is often less. When the diameter of the stalk diminishes below 4 micra, it does so in local areas only (*cf.*, Fig. 14*g*, *h*, *i*).

Amoeboid activity and cleavage activity

Many workers have noted that the polar surface of the cell bubbles actively during cytokinesis (Bowen, 1920—in *Euchistus spermatocytes;* and Lewis 1942—in tissue culture fibroblasts). This is not the case with the egg of the sea urchin during the first cleavage, instead the polar surface remains smooth and inactive. However, a variety of agents, will cause the formation of blebs in the sub-furrow region. One such agent is hypotonic calcium-free sea water. The blebs usually begin to form after the completion of the major furrowing and they give rise to sizable spherules which are cut off by a process very much like cleavage (Figs. 46*a*, *b*, *c*). The inactivity of the polar surface may indicate that the cortex there is different from the equatorial cortex in Arbacia.[2]

Eggs that have been in the hypotonic medium for some time may show a sudden rush of endoplasm from one blastomere to the other, often causing the blastomeres to become very unequal in size (Fig. 47 and Figs. 35 to 39).[3] This endoplasmic flow is a very rapid one, usually lasting only two or three seconds. It is remarkable, however, that the flow is accompanied by a rapid deepening of the furrow, appearing as though a tension has been suddenly overcome, allowing the furrow to constrict much more rapidly than usual. The sub-cortical flow in such eggs may be down one side of the furrow, through the constricted stalk and up the other side of the furrow, yet the furrowing continues normally to completion (Fig. 47).

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[2] The view that there is a special substance (a special type of plasmagel) located around the equator has been espoused by a number of workers, Marsland (1942) and Lewis (1942) among others. Beams and King (1937) are of the opinion that they have removed the "surface active material" of Ascaris eggs by centrifugation at 150,000 × gravity.

[3] The rush of endoplasm described is in this case related to cleavage. It resembles the amoeboid activity described by Moser (1940) after urea treatment. Moser, p. 77, cites other cases from the literature.

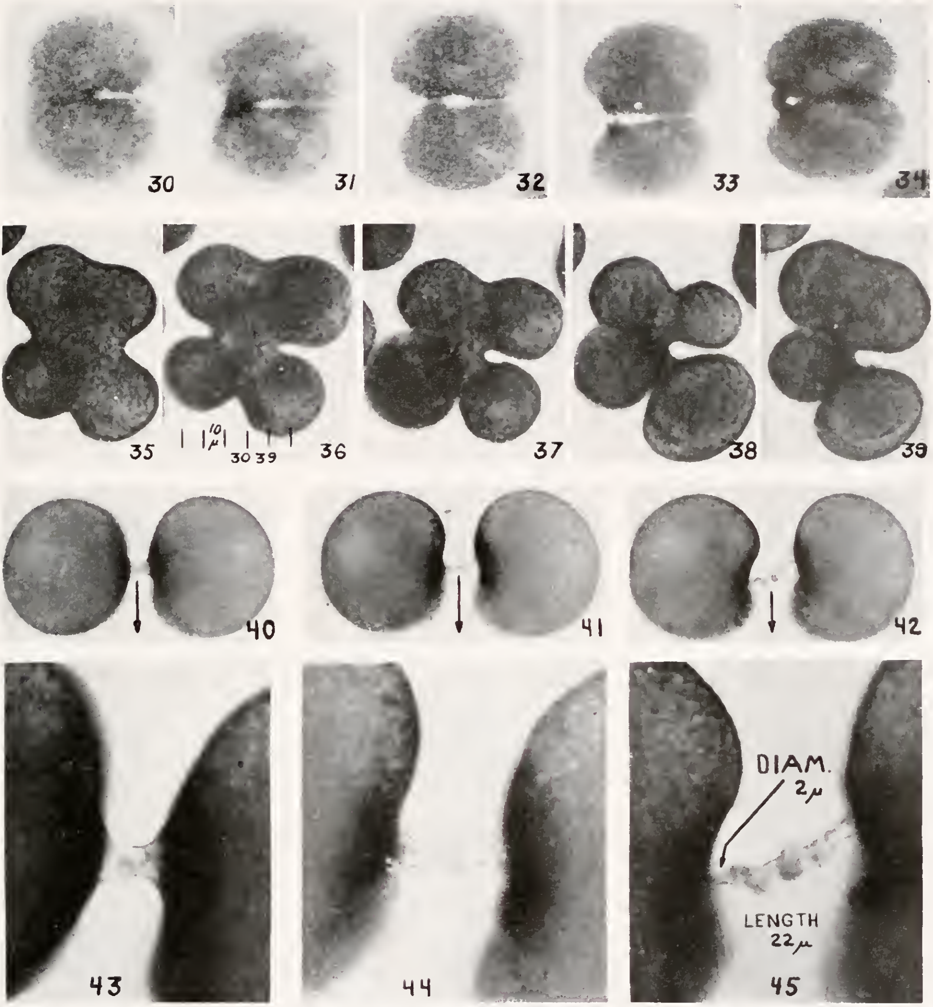
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PLATE III

FIGURES 30, 31, 32, 33, AND 34. Eccentrically placed cleavage stalk. Eggs in fertilization membrane at 10° C., in calcium-free sea water.

FIGURES 35, 36, 37, 38, AND 39. Volume changes of individual blastomeres. Calcium-free sea water.

FIGURES 40, 41, AND 42. Elongation of the cleavage stalk; in 65 per cent sea water at room temperature. Time intervals: Figures 40–41, 1 min. and 40 sec.; Figures 41–42, 1 min. and 35 sec.

FIGURES 43, 44, AND 45. Enlargements of Figures 40, 41, and 42. The edges of the nearly transparent stalk have been retouched in Figures 4, 8, 26, 27, 28, 43, 44, and 45.

Discussion

The stalk

The occurrence of a stalk during the cleavage of the Arbacia egg is correlated with the degree of gelation of the furrow cortex. Both the observations made in this paper and those of other workers who have concerned themselves with the degree of gelation of the egg cortex confirm this. The results of several workers are summarized below:

Brown (1934): Cortical pigment granules are especially resistant to displacement by centrifugation during the division phase.

Chambers (1938): The furrow cortex resists disintegration after the two incipient blastomeres have been punctured at the poles.

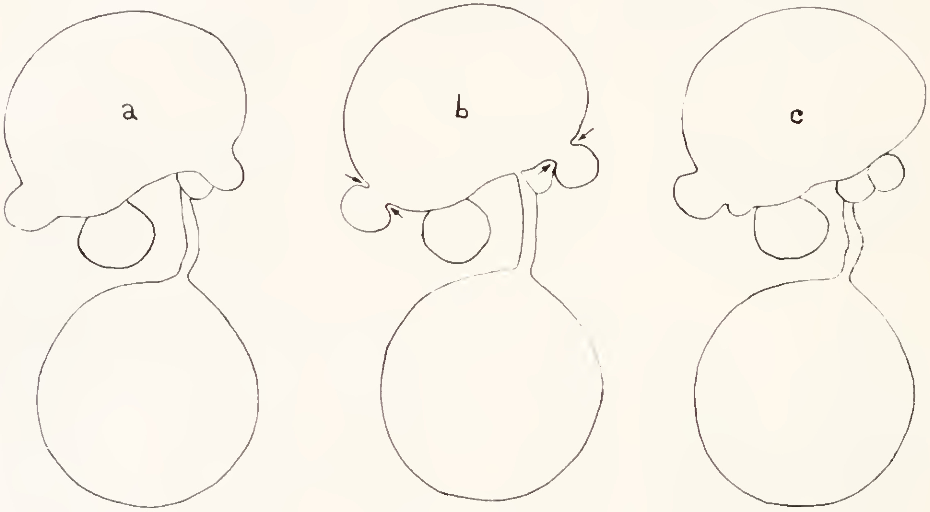

Figure 46

Brown and Marsland (1936): There is a quantitative decrease in the gel value of dividing eggs as the hydrostatic pressure is increased. Under high pressures the furrow regresses.

No one has yet recorded the effect of temperature, hypotonicity and lack of calcium ion upon the cortex of the dividing Arbacia egg, although these observations have been made upon the unfertilized egg. A brief summary of this work follows:

Costello (1938): It takes progressively longer to fragment the eggs as the temperature is lowered.

Cole (1932) and Harvey (1943): It takes longer to fragment eggs in hypotonic than in isotonic solutions.

Harvey (1945): Arbacia eggs break less readily in solutions possessing calcium ions than in solutions lacking calcium ions.

These treatments (low temperature, hypotonicity and calcium ion) are precisely the ones which favor the development of a cleavage stalk. It is possible that these treatments may increase the elastic strength of the egg surface by toughening the extra cortical structures, but it is probable that they favor cortical gelation as well.

Hypotheses concerning the mechanism of cleavage; surface tension

Chambers and Kopac (1937) found that clean oil drops of the proper interfacial tension with sea water, will coalesce spontaneously with a naked egg (Arbacia, Lytechinus, and Echinometra). They state: "The tendency to coalescence in the furrow and polar zones of cleaving eggs (late amphiaster and later) was investigated and no difference was found." They used oils whose approximate tensions in contact with sea water were 30, 10, and 3 dynes per cm. The fact that coalescence occurs at all indicates a fluid layer around the egg periphery. Spontaneous coalescence does not occur in *Amoeba proteus* (Kopac and Chambers, 1937), which indicates a non-fluid surface. In view of these observations any surface tension hypothesis is untenable.

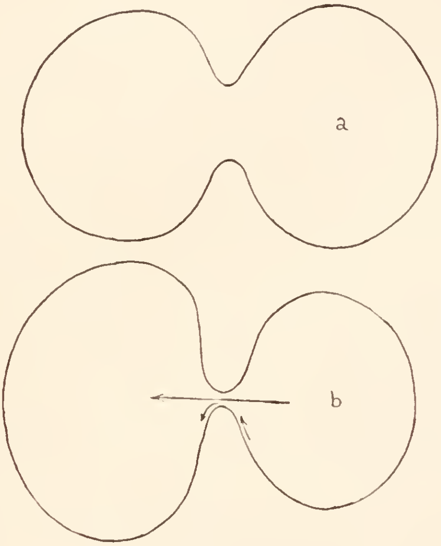

FIGURE 47

Subcortical currents

Chambers (1938) has hypothesized that cleavage results from the activity of "the sub-cortical currents (which) sweep around the two asters and add gelating material to the inwardly growing cortex." In this hypothesis he combines his own observations with those of Schechtman (1937) on localized cortical growth during the cleavage of the amphibian egg. It was shown in the present paper (page 280) that normal furrowing may be associated with abnormal currents, which argues against the importance of such currents for division; moreover Lewis (1942) found no currents in the dividing fibroblast.

Astral cleavage

An astral theory of cleavage, much modified from Gray (1931), has been elaborated by Katsuma Dan (1943). He believes that the asters are composed of radiate fibers with intrinsic rigidity; he considers them to be anchored to the cortex; he believes that the rays cross at the equator; and he believes that the spindle elongates autonomously. The following quotation (Dan 1943) summarizes his theory of cytokinesis: ". . . it was also shown that this concept of the mechanism of cell division is adequate to explain the stretching phase of the furrow surface. That is, when two such radiate asters are pushed apart, they can in turn, push the cell membrane of the polar region somewhat as a paint brush would push some object. As they travel away, however, since they enclose the fluid endoplasm within the interspaces of their rays, the fluid endoplasm is carried away from the equatorial region and the cortex there is sucked in, giving rise to a furrow. The cortex is stretched as it is pulled in by the suction."

The strength of Dan's hypothesis lies in its ability to explain the differential stretching and shrinkage of the surface which he and his coworkers observed (Dan, Yanagita, and Sugiyama, 1937; Dan and Yanagita, 1938; Dan, 1943) and for which no other explanation has been forthcoming. It appears that Dan's hypothesis will explain such unusual cleavages as are pictured in Figures 9 and 10 of the present paper. It could be assumed that one element of the tripolar spindle elongated before the others causing the asters to move apart, and by the suction mechanism, causing the development of the initial furrow (Fig. 9 at *a*). On this assumption the development of the other furrows (Figs. 9*b* and *c*) begins later, presumably because the other two spindles begin their elongation later. The secondary furrow (Fig. 10 at *a'*) is presumably caused by the suction resulting from the separation of the lower two asters. Similar explanations would doubtless serve for the tetra-astral cleavages shown in Figures 23 and 24 of the present study. One can imagine also that the crossing rays from all four asters, if they became attached to the cortex, would explain the flattening of the upper and lower surfaces of the egg observed in Figure 15.

Dan's hypothesis is not in accord with the observations presented here concerning the continued elongation of the cleavage stalk in hypotonic sea water for it is impossible to see how the astral suction mechanism could explain the further constriction of a long, completely gelated stalk.

The main weakness of the astral suction hypothesis lies in its limited scope. It fails to explain undoubted cases of anastral cleavage (tissue culture, for example) frequently noted in the literature. Dan's easy conclusion that all of these anastral cases are explainable by his astral suction hypothesis (". . . it is possible to imagine that in cells of the anastral type, similar gelation systems may be existing although they cannot be discerned morphologically") is unconvincing.

One of Wilson's observations is discordant with Dan's hypothesis. Wilson observed, in a form which normally has asters, that a spindle need not be present for complete cleavage to occur. He found that a cleavage furrow may cut in around the base of an isolated aster and result in a complete cleavage. Compare Wilson (1901), page 376 and Figure 11.

In one of Chamber's microdissection experiments he bisected the partially cleaved egg in a plane at 45° to the plane of the furrow (1924, Fig. 36). The cut resulted immediately in two cells. However, the original furrow remained on each artificially produced blastomere and, on each, the furrow gradually cut through forming two small "cells" as well as two large ones. This continued cleavage seems to be quite unexplainable by Dan's hypothesis which requires crossed astral rays, an elongating spindle and a suction produced by the separation of the asters.

Cortical growth or cortical contraction?

Schechtman has proposed another theory of the mechanism of cytokinesis. He suggested (1937) that the furrow cortex grows by the "intussusception of clear cytoplasm," but simple growth of the equatorial cortex would not be expected to cut the egg in half. Other factors must account for the inwardly directed furrow and its narrowing. It seems clear that there is a stretching of the egg cortex at the time of furrowing as concluded by Dan et al. (1937, 1938), by Schechtman (1937) and by Motomura (1940), but whether the stretching is active (the result of growth) or whether it is passive and due rather to a contracting ring at the head of the furrow (Lewis, 1942), is not easy to decide. Schechtman is of the opinion that "Cleavage is initiated by a contraction of the egg cortex at the site of the future furrow." And he notes that the "Cortex becomes thicker and bulges toward the egg interior." He therefore uses both contraction and cortical growth in his complete hypothesis. The observations made in this paper on the continued constriction of small stalks after they consist entirely of gelated material are taken as strongly favoring the constricting ring theory of Lewis. For if the gelated stalk is able to contract at that late stage of cleavage, it seems reasonable to suppose that it possesses contractile power earlier. The direction of contraction is ringwise about the equator (Fig. 29*a*) and it is to be expected that such contraction would draw stained areas out into fine lines as Schechtman observed, if such areas are located in the furrow or subfurrow region.

It would be illuminating to know whether or not kaolin particles placed *around* the equator would be brought closer together during the furrowing but no one has made these observations.

Amoeboid activity and bleb formation

One can scarcely observe the amoeboid behavior of eggs in hypotonic media and particularly the "normal" false cleavages which occur during the amoeboid phase preceding pronuclear fusion in the nematode egg (Spek, 1918), without being convinced that a fundamental similarity exists between amoeboid motion and cleavage. Moreover the abscission of blebs is strikingly similar to cleavage.[4] It is suggested that any deforming force which establishes an isthmus about the cell or a part of the cell will result in the development of a contracting ring disposed around the isthmus, provided that the egg is in the cleavage phase. This view would explain why the normal egg, deformed by the elongating spindle, cleaves at the equator. It would explain why cleavage planes cut in around the base of cytasters which are unconnected to a spindle (Wilson, 1901) and it would explain why blebs formed in the sub-furrow region may cut off from the remainder of the egg as reported above. This hypothesis also agrees with the idea that the enlarging gelated asters play a mechanical role in localizing the furrow.

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[4] Very recently Holtfreuter (1946) has suggested "that in normal cytoplasmic division the activity of the nucleus and of the endoplasm are of a mere secondary importance." He observed that isolated, embryonic amphibian cells may develop annular constrictions which lead to the fragmentation of the cell. He considers, however, that the contraction occurs in the membrane rather than in the plasmagel layer.

SUMMARY

1. Under certain conditions the eggs of *Arbacia punctulata* develop a cleavage stalk between the first two blastomeres. No stalk forms in sea water if the temperature is in the 20° C. to 30° C. range; low temperature (10° C.) causes the development of a stalk in sea water; a short stalk develops in isotonic calcium-free sea water at 20° C.; a very long stalk develops if eggs are cleaving in hypotonic sea water (65 per cent).

2. The effect of the above treatments on the appearance of cleaving dispermic eggs is described.

3. Evidence indicates that stalks of 8 micra diameter are all gel, yet in hypotonic sea water they continue to constrict and elongate. This is good evidence that the cortical gel has inherent contractile properties.

4. It is hypothesized that any event which deforms the Arbacia egg (if it is in the "cleavage phase") leads in some way to an orientation of contraction around the isthmus. The deforming force may be an enlarging aster, an elongating spindle, or an endoplasmic flow.

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DEVELOPMENTAL RELATIONS BETWEEN GENITAL DUCTS AND GONADS IN DROSOPHILA

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While studying the reproductive system of *Drosophila simulans* gynandromorphs Dobzhansky (1931) made the following interesting observation: If female genital ducts and testes were present in the same individual and if the female ducts were attached to the testes, the latter underwent extreme degeneration. Yet, the attachment of male genital ducts to ovaries did not affect the development of these organs.

Now we know that in normal development the attachment of the ducts to the gonads takes place during the early period of pupal life. We know further that by transplantation of gonads from one individual to another it is possible to obtain attachment of the transplanted organ to the host ducts. This knowledge makes it possible to attack experimentally the question whether the degeneration of testes when attached to the female ducts, as observed in *Drosophila simulans* gynandromorphs, is a peculiarity of this special case, or whether the phenomenon is a general one and always occurs when female ducts and testes are brought into contact with each other.

The problem can be approached experimentally in two ways: the larval testes can be transplanted into female host larvae or the female genital disc from which the ducts originate can be transplanted into male larvae. By transplanting two or three testes into one host, the chance that one transplantal will attach itself to the host duct is quite good. The chances for attachment of the testes to the female duct are even better when the six oviducts which arise by outgrowths from the three transplanted imaginal discs compete with the two host ducts for attachment. In the following studies both these methods were used.

EXPERIMENTAL

Transplantation of testes into female ducts

Two or three testes of mature virilis larvae were transplanted together into the abdominal cavity of female host larvae of the same age. After the hosts had emerged, the condition of the transplants and their relationship to the female genital system was studied in careful dissections. This series consisted of ten cases. The following was found. All transplants had failed to assume their characteristic spiral shape. This was to be expected, since the work of Dobzhansky (1931) and Stern (1941a and b) had shown that the testes have to be attached to the vas in order to accomplish their spiral growth. In six out of ten cases one of the transplanted testes had attached itself to one of the oviducts of the hosts. The attached testis was always greatly reduced in size and appeared degenerate. Figure 1 shows camera lucida drawings of five representative cases of this series. It will be noted that the degenerative reduction occurs only when the testis is attached to the *oviduct* of the host (Fig. 1*A*, *B*, *D* and *E*). Testes that lie free in the body cavity (Fig. 1*A* and *B*) or testes that have been attached to the ovary (Fig. 1*C* and *B*) are unaffected. Thus the principle which produces degeneration is apparently given off only by the oviducts and depends for its action on a close cellular contact with the testes. This principle, moreover, seems unable to penetrate larger cell barriers, for testes which were connected to ovaries which in turn had their normal oviduct connection remained unaffected (Fig. 1*C*). Yet two testes which had established close contact with each other had both suffered degenerative reduction, although only one of these fused organs has actually established contact with the oviduct (Fig. 1*A* and *E*).

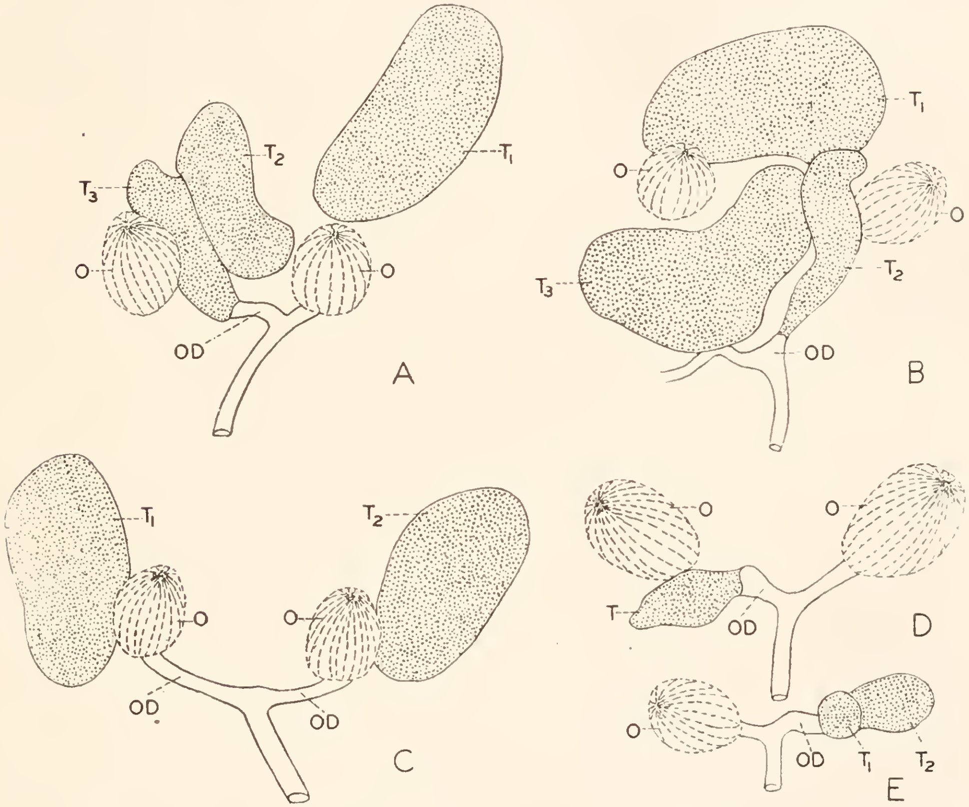

FIGURE 1. The spatial and developmental relations of transplanted testes to the reproductive system of their female hosts. *O*, ovary; *OD*, oviduct; T_1 to T_3, transplanted testes.

Transplantation of female genital discs into male hosts

In a second series of experiments, two or three female genital discs from mature virilis larvae were transplanted together into the body cavity of hosts of the same age. The condition of the host testes and their relationship to the transplanted female structures was again studied by dissection. Several of the affected testes were also sectioned and studied histologically. Thirty successful cases were available for investigation. In seven of these cases, the host testes were not connected to the transplanted ducts, although the latter had developed well and were found in the immediate neighborhood of the male gonads. The testes of these seven hosts were normal in size, shape, and histology. One testis in each additional animal was not connected with the vas of the host, nor to any of the transplanted ducts. These testes were not coiled but were otherwise normal. The other testis in two of these individuals was connected to the vas of the host and was normal, while the other testis of the third individual, although connected to the vas, was not coiled. The non-coiling of an attached testis is rare, but has been observed at times in otherwise normal animals. Whether the inability of attached testes to coil is a result of faulty connections with the vas or whether the vas in these cases has lost its growth inducing capacity is not known.

TABLE I

Transplantation of female genital ducts into male hosts

Number of discs transplanted	Testis free (round)		Testis (spiral). Normal attached		Testis attached to ♀ duct		State of degeneration of testis when attached to ♀ duct	
	One side	Both sides	One side	Both sides	One side	Both sides	One side	Other side
3			Yes*		Yes		+	
3			Yes		Yes		++++	
3			Yes		Yes		++++	
3			Yes		Yes		++++	
3				Yes				
3				Yes				
3			Yes		Yes		++++	
2			Yes		Yes		+++	
2			Yes		Yes		++++	
2						Yes	+++++	+++++
2	Yes		Yes				+	
3			Yes		Yes		++	
3				Yes				
3			Yes		Yes		++++	
3			Yes		Yes		++++++	
3			Yes		Yes		+++++	
2				Yes				
2			Yes		Yes		+++	
2		Yes						
2		Yes						
2		Yes						
2				Yes				
2				Yes				
2						Yes	+++++	+++++
2			Yes		Yes		++++	
2						Yes	+++	+++++
2 h.				Yes				
2 h.			Yes		Yes		++++	
2 h.			Yes		Yes		++++	
2 hv.			Yes		Yes		++++	

h. = hydei discs into hydei hosts. hv. = hydei discs into virilis host. * = testis attached to vas, but not spiral.

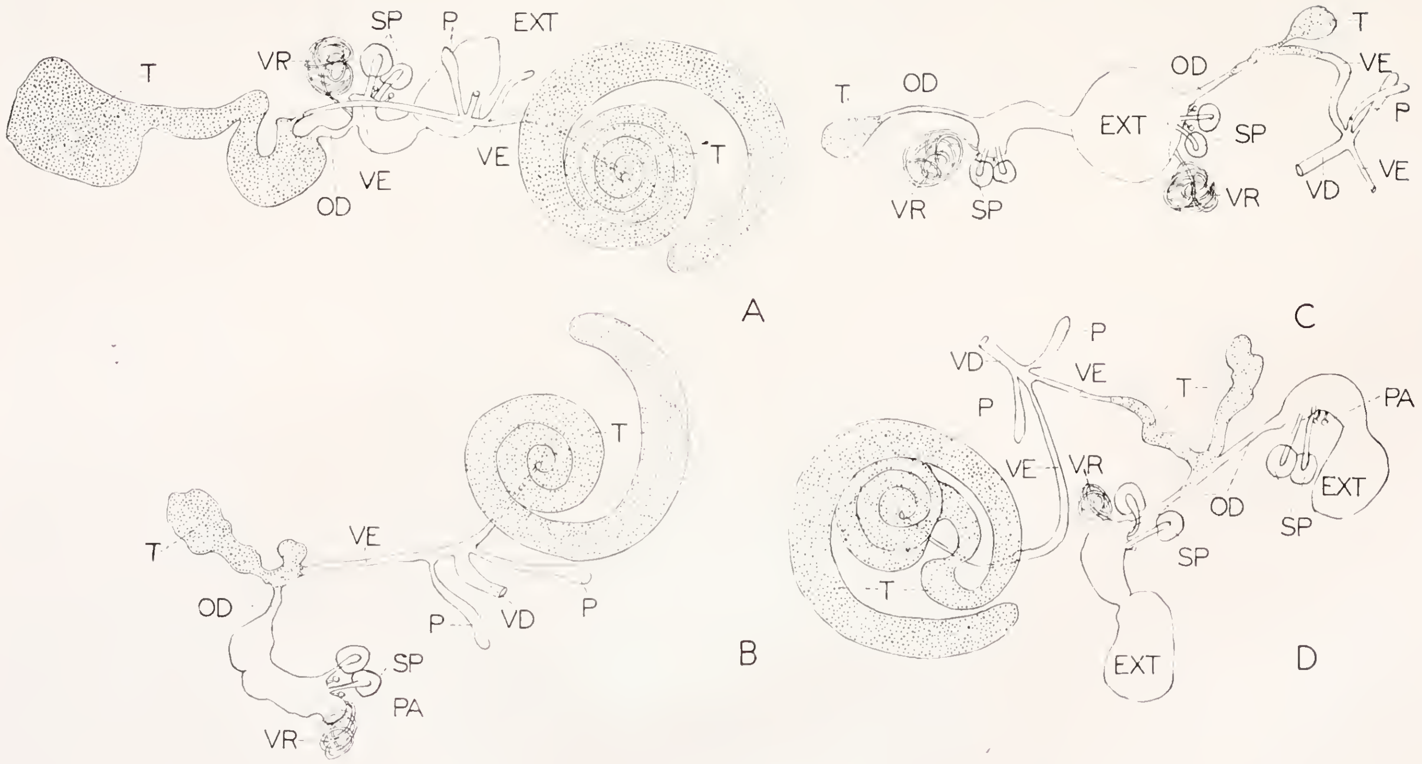
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FIGURE 2. The spatial and developmental relations of transplanted female genital structures to the reproductive system of their male hosts. *EXT*, external female genitalia formed by the transplants; *OD*, oviduct; *P*, paragonium; *PA*, parovaria; *SP*, *spermathecae; T*, testis; *VD*, vas deferens; *VE*, vas efferens; *VR*, ventral receptacle.

In twenty individuals one or both testes were connected to the oviduct of the transplant. In some cases one testis was even found to have connection with the oviducts of two discs. All testes that were attached to oviducts showed a more or less pronounced degree of reduction and appeared degenerate. Table I summarizes the results of this experimental series. The state of degenerative reduction of the testes in this table is indicated by crosses. One cross signifies slight; six crosses, extreme size reduction. Figure 2 shows camera lucida drawings of four representative cases of this series. Figure 2*A* is a case listed in Table I having one cross. Figure 2*C* is listed by having six crosses and Figure 2*D* and *B* are listed by having five crosses each. Figure 3 illustrates by microphotography an extremely reduced testis.

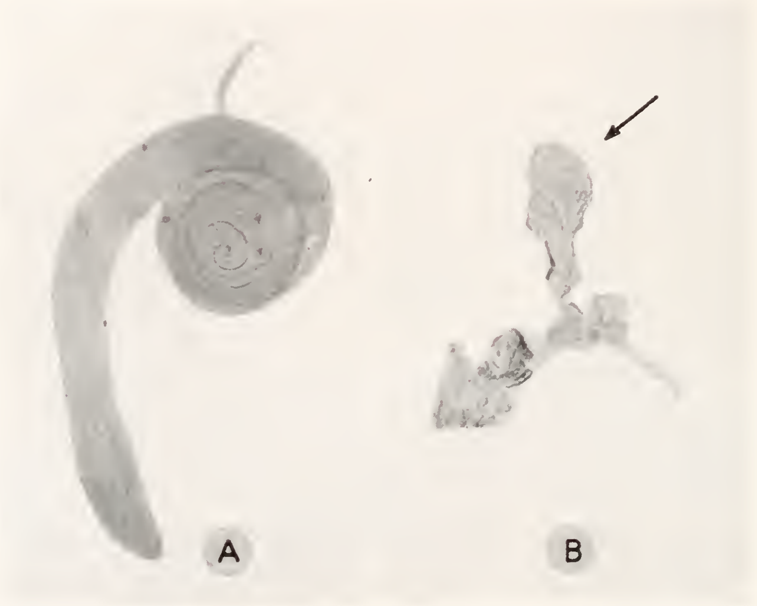

FIGURE 3. *A*, normal adult testes. *B*, (arrow) testis of the same individual degenerated under the influence of a transplanted and attached oviduct.

It will be noted from Table I that the reduction of the testes is in most cases very pronounced. Only six of the testes attached to oviducts were reduced to state "3" or less, while sixteen testes were degenerated to state "4" or more.

This experimental series thus confirms strikingly the original observation that testes attached to female oviducts suffer degenerative changes and that it is the oviduct that elicits the principle causing degeneration. The observation from the previous experiments that the presence of oviducts *per se* has no effect on testis development is also confirmed, for testes in the presence of as many as three pairs of oviducts in the immediate organic environment remained normal if they were not attached to the oviduct. In comparing the attached testes of the two series with each other, a difference in their general shape was noted. While the transplanted testes in the first experimental group were small, roundish bodies, the testes in the second experimental group were in most cases thin and elongated in shape (compare Fig. 1 with Fig. 2). Now it was found in the second group that in all cases when the attached testis was thin and elongated it was attached not only to the transplanted oviduct but also to the vas efferens of its host (Fig. 2). In those cases, however, where the testis attached only to the transplanted oviduct, had not established connection with the vas, it was roundish. This situation is well illustrated in Figure *2C*. The left testis in this case, a small roundish degenerated organ, is attached only to the oviduct while the right testis, which is attached to the vas efferens of the host and to one transplanted oviduct, has become an irregularly elongated structure. The elongated shape of such a degenerate testis is thus due to the stimulating influence of the vas on the growth of the testis, which in normal development leads to the coiling of this organ, while the observed degeneration is caused by the influence of the oviduct.

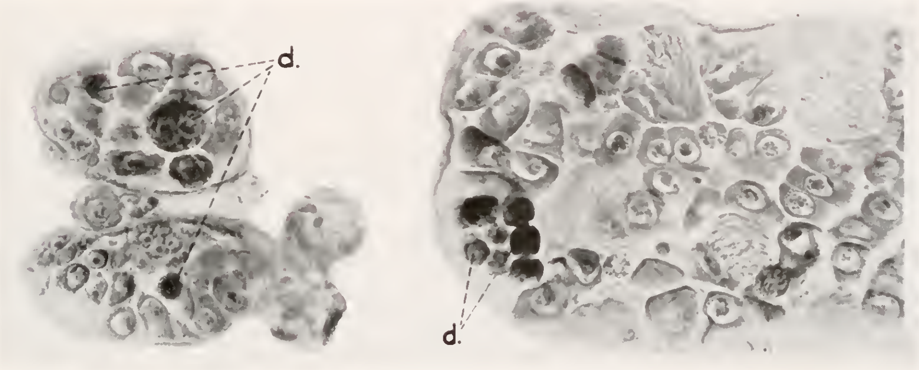

FIGURE 4. Sections through two extremely reduced testes. *d*, degenerating cells.

Not only virilis but also hydei oviducts cause degeneration of hydei testes attached to them. This was shown by three cases in which larval hydei female discs were transplanted into hydei male larvae (see Table I).

The factor in the oviduct causing degeneration of the testis by contact is not species specific, for hydei oviducts will cause virilis testes to degenerate (see Table I).

Sections of reduced testes were made and their histology studied. It was found that, depending upon the degree of reduction of size, the testes contained various amounts of spermatogonia and spermatocytes in all stages of degeneration. The remnants of disintegrated cells in the form of granular picnotic masses together with quite normal appearing cells were observed. Figure 4 shows the condition found in an extremely reduced testis.

CONCLUSION AND SUMMARY

By transplanting female genital discs into male hosts, attachment to the host testes of oviducts developed from transplanted genital discs is obtained. In these cases the attached testes suffer extensive degeneration. Only cellular contact of the oviducts to the testes brings about this phenomenon. Unattached female ducts do not affect the development of the testis. The principle causing degeneration is not species specific. The findings indicate that the phenomenon encountered is no unique instance, but representative when oviduct and testis establish cellular contact during pupal development.

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A HISTOLOGICAL STUDY OF SYNDISYRINX FRANCISCANUS, GEN. ET SP. NOV., AN ENDOPARASITIC RHABDOCOEL OF THE SEA URCHIN, STRONGYLOCENTROTUS FRANCISCANUS [1]

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INTRODUCTION

Up to the present time eight genera of worms endoparasitic in echinoderms and sipunculids have been described that belong to the rhabdocoel family Umagillidae Wahl, 1910b. Schneider described the first species, *Anoplodium parasita,* in 1858. Since then six questionable and three valid species of this genus have been reported from widely separated localities as parasites of holothurians. Their distribution extends from the Mediterranean, Ionian, and North Seas to Japan and the Philippines (Bock, 1926). *Syndesmis echinorum* Francois, 1886, the only species of the genus, is found in echinoids. It has been collected in the Mediterranean (Russo, 1895), Norway (Westblad, 1926), and the English Channel (Braun, 1889). Three species of the genus *Collastoma* are found in sipunculids at Roscoff (Dörler, 1900), the Gulf of Kola (Beklemischev, 1916), and the Bay of Naples (Wahl, 1910a). The genus *Desmote* is represented by one species, *D. vorax,* discovered in a crinoid collected in the Gulf of Kola (Beklemischev, 1916). A single species parasitic in holothurians has been described in each of four genera, i.e., a Japanese form, *Xenometra arbora* Ozaki, 1932, and three reported from the coast of Norway, *Wahlia macrostylifera* Westblad, 1930, *Anoplodiera voluta* Westblad, 1930, and type genus *Umagilla forskalensis* Wahl, 1909.

The only reference to a member of the Umagillidae from the Western Hemisphere was made by Powers in 1936. He reported the presence of a Syndesmis-like worm in the coelomic cavity of the echinoid, *Centrechinus antillarum,* at Tortugas. A complete description was not given; however, as compared with *Syndesmis,* noticeable differences were observed in details of the copulatory apparatus and the arrangement of the shell glands. While the endoparasitic rhabdocoel of *Strongylocentrotus franciscanus,* the large common sea urchin of the California coast, is well known to some investigators who have worked at Pacific Grove, a description of this worm has not been recorded in the literature prior to the present account.

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[1] This work was done at the Wilson Zoological Laboratory of the University of North Carolina in partial fulfillment of the requirements for the degree of Master of Arts. The author is indebted to Professor D. P. Costello for suggesting the problem, for the slide preparations upon which this study was essentially based, and for the invaluable suggestions and criticisms rendered during the preparation of this paper. The author wishes to acknowledge his appreciation to Dr. L. H. Hyman for many valuable recommendations and for permission to introduce her revised and hitherto unpublished terminology relating to this group. To Miss Catherine Henley the author expresses his gratitude for the translation of a number of the references cited herein.

[2] Now in the School of Biological Sciences of Stanford University.

Systematic position[3]
Order Rhabdocoela
Suborder Lecithophora
Section Dalyellioida
Family Umagillidae Wahl, 1910
Subfamily Umagillinae Wahl, 1910
Genus Syndisyrinx, gen. nov.
Genotype Syndisyrinx franciscanus, sp. nov.

Holotype. A whole mount in the United States National Museum, Washington D. C.

Repositories of type material. In each of the following repositories a whole mount, a transversely sectioned, and a sagittally sectioned preparation selected from the type material have been deposited: U. S. National Museum, Washington, D. C.; American Museum of Natural History, New York City; British Museum, London; California Academy of Science, San Francisco; Wilson Zoological Laboratory of the University of North Carolina; and Museum of Natural History, Stanford University. Additional preserved material may be obtained from the author or from any of these institutions.

Type locality. Mussel Point, Monterey Peninsula, California, Lat. 36°, 37′, 20″ N., Long. 121°, 54′, 15″ W.

Collectors. D. P. Costello, 1937 and H. E. Lehman, 1945.

Distinguishing characteristics. Umagillinae with a single intestine, paired and branched ovaries, cuticular penis, and a bursa seminalis connected by cuticular ducts to the seminal receptacle and bursal canal.

Materials and Methods

Fifty-four rhabdocoel parasites were obtained from two specimens of the sea urchin *Strongylocentrotus franciscanus* (A. Agassiz) by Dr. D. P. Costello in August 1937 at Pacific Grove, California. These specimens were fixed in Heath's, Boveri's, Lillie's and Worcester's solutions. Five of the individuals were sectioned serially at $10\,\mu$ and stained with Heidenhain's iron hematoxylin and orange G. One of these preparations was exceptionally fine and the majority of the accompanying figures were made from it. Unfortunately this preparation, which the author intended to designate as the holotype, was lost when a microscope was stolen. This material, including the slide preparations, was turned over to me by Dr. Costello. The morphological study was based on this material.

In the summer of 1945 during June, July, and August, the author collected several hundred additional specimens from the same locality. Over sixty urchins were examined and all were found to be infested; frequently three dozen or more parasites were obtained from the intestine of a single host. These worms were fixed in Heath's and Beauchamp's solutions. Seventy were sectioned serially at $10\,\mu$ and stained with Mayer's acid hemalum and triosin. Thirty whole mounts stained with paracarmine were also made. The type material was selected from these prepparations. At this time another parasite of *Str. franciscanus* was discovered which differed from *Syndisyrinx* in shape, manner of locomotion, and color. A description of this worm is being prepared and preliminary examination of sectioned material indicates a close relationship to *Syndesmis echinorum.* Upon the suggestion of Prof. A. R. Moore, who had occasionally observed parasitic worms in *Str. purpuratus* (Stimpson), forty-seven of these urchins were examined. In twenty-nine of them, worms that are very similar to, and may be identical with *Syndisyrinx franciscanus* were present in small numbers.

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[3] Classification according to Bresslau (1933), with the exception of "Family Anoplodiidae Graff, 1913," which has been rejected in favor of "Family Umagillidae Wahl, 1910b," inasmuch as no reason is given by Graff for discarding the older name or for selecting *Anoplodium* as type genus. The subfamily Umagillinae has been retained as designated by Wahl, 1910b.

General Morphology

The living animals are bright red with a dark brown or yellow median longitudinal line which marks the extent of the intestine. The worms are flattened dorsoventrally and have a leaf-like appearance, being rounded at the anterior end and slightly pointed posterad. Individuals vary in size from 2 to 3 mm. long and 1.6 to 2.5 mm. wide. The body is thickest at approximately one-fourth of the distance from the anterior end and at this level measures about 0.5 mm. in the dorsoventral axis. Laterally and posteriorly the thickness of the body diminishes gradually to about 0.2 mm. at the periphery. A ciliated epithelium covers the entire surface; rhabdites and cuticle are lacking.

The mouth is situated on the ventral surface about one-fourth of the distance from the anterior end and a common genital pore opens ventrally at the posterior extremity of the body. The musculature and parenchyma are typical of other Umagillidae. No excretory system was observed. The strongly muscular pharynx is typically doliiform and possesses pharyngeal glands; it communicates by a short oesophagus with the gut. The intestine, possessing a number of small lateral diverticula, extends posterad under the dorsal epidermis along the mid-line and terminates one-quarter of the distance from the posterior end of the body. The gut contains no permanent lumen and food masses lie in temporary cavities surrounded by large digestive cells. The brain, composed of two cerebral ganglia connected by a wide commissure, lies anterior to the pharynx and gives off paired anterior, lateral, and posterior nerves.

Lobed testes lie lateral to the mid-line in the anterior half of the body. Accessory glands empty into the sperm duct that arises from each testis and passes anterad. These paired tubes unite mesially and enter a small spermiducal vesicle that is continued posterad as a muscular common sperm duct which lies dorsal to the uterus along the mid-line. This tube terminates in an elongated cuticular stylet, the penis, which is enlarged and funnel-like at the base. The penis stylet enclosed in the male antrum extends through the posterior third of the body to the common genital antrum and over most of its length does not exceed 3 μ in diameter.

Paired vitellaria are found immediately posterior to the testes; they are greatly ramified and fill most of the ventrolateral spaces in the middle third of the body. Posterior to the vitellaria a pair of ovaries is located, one on each side of the mid-line. Laterally each branches into five or more finger-like lobes. Three or four collecting ducts from the vitellaria empty with the ovaries and seminal receptacle into the anterior end of the ovovitelline duct. The seminal receptacle is oval and filled with sperm. Located posterodorsad to this organ is a vesicular, sperm-filled bursa seminalis connected to the seminal receptacle by a fine cuticular insemi-

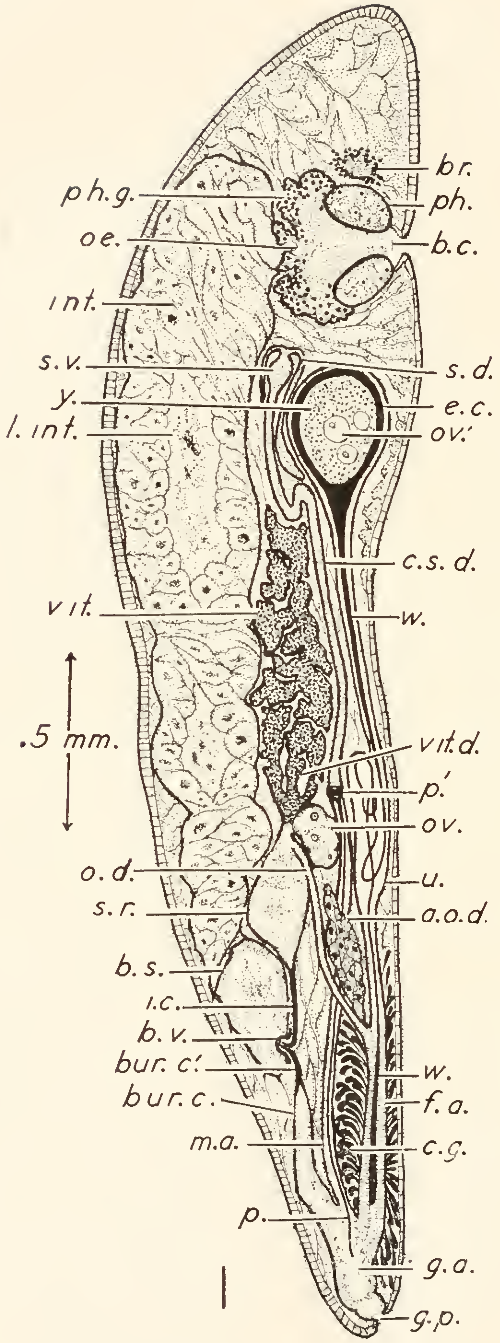
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FIGURE 1. Semidiagrammatic median sagittal section.

nation canal. Arising in close association with this tubule is a similar duct, the cuticular proximal part of the bursal canal that passes posterad from the bursa seminalis approximately 60 μ before widening into the posterior muscular portion of the bursal canal (vagina). A cuticular sheath surrounds the openings of these two ducts into the bursa seminalis. The composite structure, consisting of this sheath and the canals passing through it, makes up the bursal valve.

An ovovitelline duct, into which accessory glands empty, arises ventrally at the anterior end of the seminal receptacle. It passes posterad and unites with the female antrum. The uterus, lying close to the ventral epidermis, extends anteriorly from the female antrum almost to the pharynx. At the anterior end of the uterus an egg capsule containing from one to five ova and numerous yolk cells is generally found. The capsule is continued posterad as a long coiled whip similar to those found in related forms. Most of the ventrolateral spaces of the posterior third of the body are filled by cement glands; they communicate by many small ducts with the female antrum. The common genital antrum is an elongated cavity at the posterior end of the body into which the female antrum enters ventrally, the male antrum and penis open mesially and the bursal canal is given off dorsally. At its posterior end is the common genital pore which opens ventrally to the exterior.

Histological Structure

Epidermis

A ciliated epithelium covers both dorsal and ventral surfaces of the body. No pigment or special gland cells were observed in this layer and a cuticle and rhabdites are lacking. The cytoplasm of the cells in the epidermal layer is granular and cell boundaries, though faintly stained, are distinct. The cells covering the dorsal surface are cuboidal and measure 10 μ from basement membrane to external surface. The cytoplasm of these cells stains moderately with hematoxylin. On the ventral surface the cells are flattened and are about 7 μ thick and from 12 to 35 μ wide; they have little affinity for hematoxylin. Cilia of the ventral epidermis are about 6.5 μ long and are almost twice the length of those found on the dorsal surface. Cells possessing the staining properties and short cilia characteristic of the dorsal layer extend for a short distance ventrally around the lateral edges. A zone 4 to 6 cells wide of intermediate nature accomplishes the transition between typical dorsal and ventral epithelium.

Musculature and parenchyma

The arrangement of the musculature is essentially the same as that described for other Umagillidae. Under the basement membrane of the surface epithelium is

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Abbreviations for Figures 1 and 2.

a.o.d.—accessory glands of ovovitelline duct, a.s.d.—accessory glands of sperm duct, br.—brain, b.c.—buccal cavity, bur. c.—bursal canal, bur. c′.—cuticular end of bursal canal, b.s.—bursa seminalis, b.v.—bursal valve, c.g.—cement glands, c.s.d.—common sperm duct, e.c.—egg capsule, f.a.—female antrum, g.a.—common genital antrum, g.p.—genital pore, int.—intestine, i.c.—insemination canal, l. int.—lumen of intestine, m.a.—male antrum, oe.—oesophagus, ov.—ovary, ov′.—ovum, o.d.—ovovitelline duct, p.—penis, p′.—base of penis, ph.—pharynx, ph. g.—pharyngeal glands, s.d.—sperm duct, s.r.—seminal receptacle, s.v.—spermiducal vesicle, te.—testis, u.—uterus, vit.—vitellaria, vit. d.—vitelline ducts, w.—whip of egg capsule, y.—yolk cells.

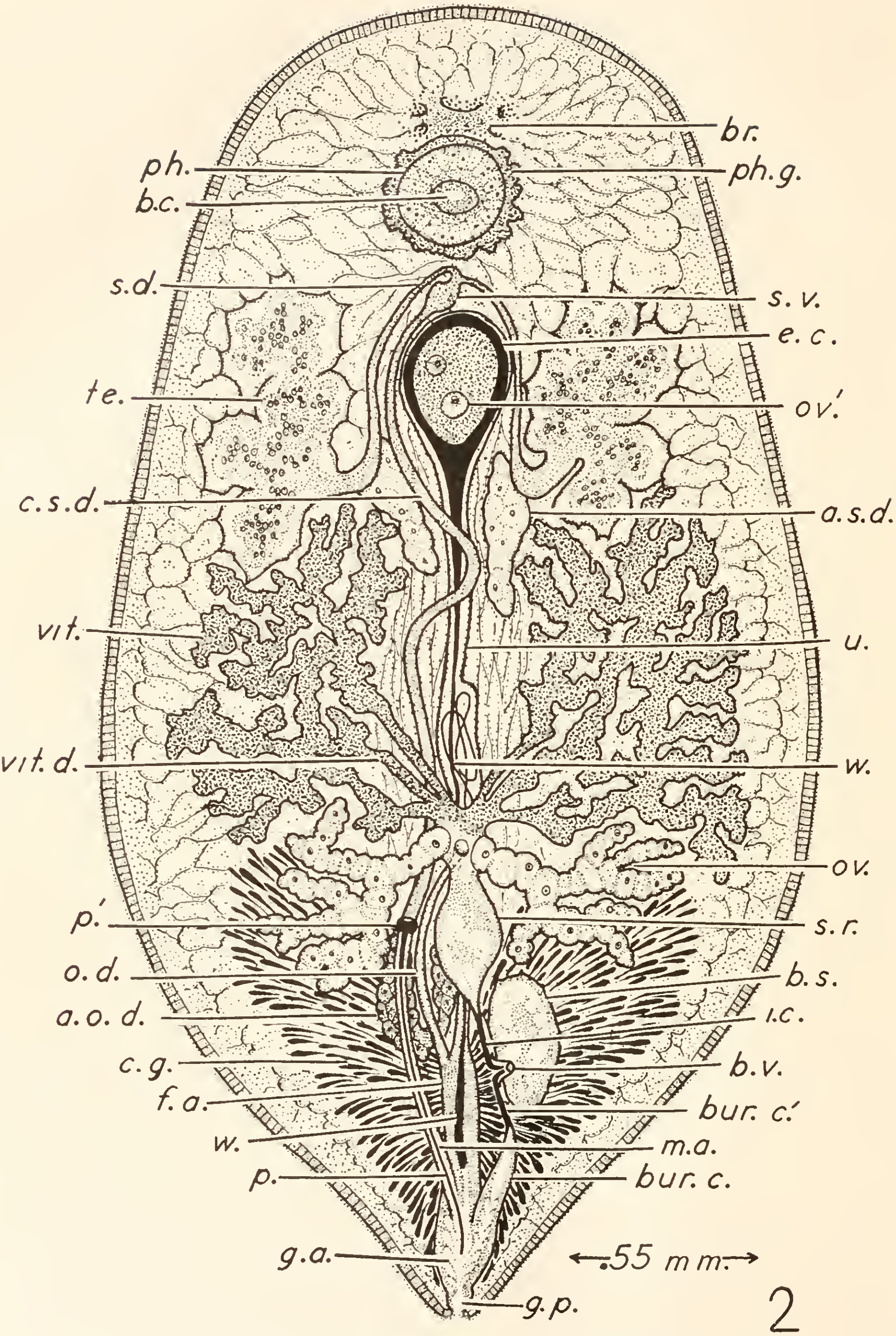
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FIGURE 2. Semidiagrammatic median frontal section, intestine omitted.

found a thin layer of subepidermal muscles (Figs. 3–5, 7, 8). The superficial muscles are circular; these overlie a longitudinal sheet, and interposed at intervals between these layers are well-developed oblique fibers. In addition to these, bundles of fibers attached to the internal organs or the basement membrane of the epidermis pass dorsoventrally through the parenchyma (Figs. 1–3). The special muscles of the reproductive and digestive systems will be described in connection with the organs with which they are associated.

A parenchyma, composed of large, irregularly shaped cells with coarsely granular or vacuolated cytoplasm, fills most of the spaces between the internal organs and epidermis. A histologically distinct parenchymatous mass of cells enclosed in a fibrous capsule extends posterad along the mid-ventral line from the posterior level of the pharynx to the region in which the female antrum enters the common genital antrum. The flattened, nonvacuolated cells of this tissue possess finely granular cytoplasm and are arranged in concentric layers around the reproductive ducts, most of which pass through the mid-ventral parenchyma (Figs. 3–5, 7). Nowhere within the parenchyma were flame cells or collecting ducts of an excretory system observed.

Nervous system

The brain is similar in all respects to those described in other members of the family. It is located just anterior to the pharynx and consists of two ganglia connected by a wide commissure. Around the central fibrous mass of the brain are numerous ganglionic cells that stain quite evenly with hematoxylin. Poorly developed anterior, lateral and posterior pairs of nerves leave the brain and can be traced for short distances into the parenchyma. No theca separates the brain or nerves from the parenchyma and no special sensory organs were found.

Digestive system

The mouth lies on the ventral surface about one-fourth of the distance from the anterior end of the body. It opens into a very small buccal cavity lined by flattened ciliated cells that are continuous externally with the ventral epithelium (Figs. 1, 8). A sphincter underlying the epithelium regulates the size of the oral opening. Lying immediately dorsal to the mouth and opening into the buccal cavity is the doliiform pharynx which has the appearance of a dorsally compressed sphere. Its dorsoventral axis is about 0.1 mm. long and its greatest diameter is about 0.17 mm. Passing dorsoventrally through the pharynx is a funnel-shaped lumen that is narrowest at the oral or ventral end. The musculature of the pharynx is similar in most details of its organization to that found in *Syndesmis* as described by Russo (1895). A thin superficial layer of vertical fibers overlies the well-defined muscles encircling the lumen of the pharynx. In addition to the circular and vertical muscles, radial fibers pass from the lumen to the peripheral surface of the pharynx. Nonmuscular cells with heavily staining reticular cytoplasm fill the spaces between the radial fibers (Fig. 8). Surrounding the pharynx is a sharply defined basement membrane to which are attached numerous short, radially arranged, protractor muscles that extend to the basement membrane of the ventral epidermis. The more oblique of these fibers serve also as dilators of the pharynx. Poorly developed retractors are attached to the equator of the pharynx and pass to the dorsal surface. Pharyngeal glands are present encircling the dorsal end of the pharynx. The peripheral contours of these glands are lobular and a thin basement membrane separates them from the parenchyma. The cells which make up these glands have indistinct cell boundaries and dense cytoplasm containing numerous granules that stain darkly with hematoxylin. Cytoplasmic continuations of the cells extend ventrally and line the lumen of the pharynx (Fig. 8). Leading dorsad from the pharynx is a short oesophagus which passes through the pharyngeal glands and opens into the anterior end of the intestine.

The intestine lies along the mid-line under the dorsal epidermis and extends posterad from the level of the brain to about one-fourth of the distance from the posterior end of the body (Fig. 1). The width of the gut varies from 0.1 to 0.2 mm. at the anterior end and diminishes gradually posteriorly. Short diverticula extend laterally on each side. The epithelium of the intestine is made up of large irregularly shaped cells containing moderately granular cytoplasm. The basal end of most cells reaches the fibromuscular investing sheath of the intestine that separates it from the parenchyma. The lumen of the intestine can only be observed when ingested material is present; this condition is similar to that found in some alloeocoels. In an animal that has been feeding, food masses often lie in cavities that have lost all direct communication with the oesophagus (Fig. 1). Food vacuoles of varying sizes are generally present in the cells surrounding the ingested material and digestive cells were occasionally observed that had apparently migrated into the food masses by amoeboid movement.

Male reproductive system

The paired testes lie lateral to the mid-line in the anterior half of the body. They are approximately 0.5 mm. long and from 0.3 to 0.5 mm. wide. Each is made up of four to six vesicular lobes, the lumina of which are in direct communication with one another (Fig. 2). Separating the testes from the parenchyma is a fibrous sheath that pentrates and partially subdivides the lobes. The chambers so formed are filled with developing germ cells and tangled masses of mature spermatozoa (Fig. 3). Mature sperm are present in all lobes but are more numerous midway between the anterior and posterior ends of the testes near the wide openings of the sperm ducts. These ducts run mesially from the testes and enter the mid-ventral parenchyma, whereupon they diminish to about 10 μ in diameter and generally continue their course anterad, dorsolateral to the uterus (Figs. 1–3). A thin epithelium surrounded by loose fibromuscular elements makes up the walls of the sperm ducts. Near the origin of these ducts from the testes, glandular cells that probably possess some accessory function are found in the mid-ventral parenchyma adjacent to the ventral walls of the tubes (Fig. 2).

At varying distances posterior to the pharynx the sperm ducts unite mesially and enter the anterior end of a common sperm duct which at this point is somewhat enlarged to form a small spermiducal vesicle (Figs. 1–3). The slightly coiled common sperm duct continues posterad from the vesicle through the mid-ventral parenchyma. It gradually diminishes in diameter from 45 μ to 12 μ. Its walls are composed of connective tissue cells surrounded by a sheath of circular, oblique, and longitudinal muscle fibers. The lumen of the tube is lined by a thin squamous epithelium that is separated from the theca by a thick basement membrane. Posteriorly, the common sperm duct unites with the enlarged base of the penis at about one-third of the distance from the posterior end of the body (Figs. 1, 2, 4). The penis lies in a muscular sheath, the male antrum, which is a diverticulum of the genital antrum. Histologically this sheath is similar in most details of its structure to the common sperm duct; however, the lining epithelium of the male antrum is thicker, in some regions almost occluding the lumen, and a thick basement membrane is lacking (Fig. 5). The copulatory organ is a cuticular tubule that extends through the posterior third of the body and is about 3 μ in thickness over most of its length. The lumen of the stylet does not exceed 2 μ in diameter except at the anterior end of the penis which is enlarged to 12 μ at its union with the posterior end of the common sperm duct (Figs. 1–3). The rim of the funnel-like base of the penis is thickened to form a collar; longitudinal muscles in the walls of the male antrum and common sperm duct attach to this collar and function as protractors and retractors of the penis.

Female reproductive system

The paired ovaries lie in the posterior third of the body. Each is made up of from five to ten lobes that branch dichotomously from common trunks arising near the anterior end of the seminal receptacle. The lobes of the ovaries are directed posterolaterad and are separated from the parenchyma by a very poorly developed theca. The branches are made up of dovetailed chains or rouleaux of compressed ova that are proliferated from primordial cells at the distal ends of the lobes (Fig. 2). Mature ova are approximately 75 μ in diameter and vary in thickness from 20 to 60 μ. The cytoplasm of immature eggs is at first homogeneous, but as development continues many small peripherally distributed granules appear that are probably stored nutrient materials. During the period of growth the nuclei of the ova increase from 7 to 25 μ in diameter and the chromatin granules gradually lose their affinity for basic dyes. In mature ova only the spherical or oval nucleolus stains deeply with hematoxylin (Fig. 4).

A pair of greatly branched vitellaria lie anterior to the ovaries and fill most of the ventrolateral spaces in the middle third of the body (Figs. 1, 2). Many of the dorsoventral muscles of the parenchyma contribute fibers to the diffuse sheath that encloses these ducts. Primordial cells at the distal ends of the branches give rise to yolk cells. As the cells increase in size, the cytoplasm which at first is homogeneous, becomes filled with refractile granules that coalesce to form amber-colored droplets (Figs. 3, 4). From each side three or four collecting ducts packed with mature yolk cells pass posterad from the vitellaria and unite near the mid-line shortly before emptying into the anterior end of the ovovitelline duct (Fig. 2).

The seminal receptacle is somewhat oval and lies ventral to the intestine within the sheath that surrounds the gut. Its anterior extremity is about one-third of the distance from the posterior end of the body. The posterior part of this organ is thin walled and masses of mature spermatozoa are observable in its extensive lumen. Anteriorly the seminal receptacle opens with the paired ducts of the vitellaria and ovaries into the ovovitelline duct which arises ventrally in this region (Figs. 1, 2). The wall of the anterior third of the seminal receptacle is lined by large gland-like cells that restrict the lumen to a narrow channel 6 to 10 μ wide which connects the posterior vesicular portion to the ovovitelline duct (Fig. 4).

The bursa seminalis lies dorsal to the vesicular portion of the seminal receptacle. It is enclosed in the same sheath that surrounds the seminal receptacle and the posterior end of the intestine (Figs. 1, 2). The large lumen of the bursa seminalis is lined by an epithelial layer very similar to that lining the posterior part of the seminal receptacle. In every specimen examined spermatozoa were found in the bursa; frequently they were aggregated into roughly spindle-shaped masses in which degenerating sperm were observable (Figs. 5, 6). Arising ventrally, or in some cases laterally, from the wall of the posterior half of the bursa seminalis is the insemination canal, a fine cuticular tubule about 4 μ in diameter connecting the lumina of the bursa seminalis and seminal receptacle. In close association with the insemination canal, a second cuticular tube of the same dimensions arises from the wall of the bursa seminalis and connects the bursa posteriorly to the bursal canal (Figs. 1, 2, 5, 6). Surrounding the ends of the ducts as they penetrate the lining epithelium of the bursa is a cuticular sheath, 7 μ in diameter and 10 μ long. The inner end of this sheath is involuted and fused to the ends of the two ducts (Fig. 6). To designate this composite cuticular structure made up of the insemination canal, the proximal end of the bursal canal and the sheath surrounding the ends of these ducts, the term, "bursal valve," is suggested.

The bursal canal (vagina) is a tubular structure about 0.1 mm. long and 20 μ in diameter that arises as an anterodorsal continuation of the common genital antrum. Its wall is composed of an inner epithelial layer surrounded by a strong fibromuscular sheath. At the posterior end of the canal the epithelium possesses cilia-like projections characteristic of the lining of the common genital antrum. Anteriorly the lumen of the canal is reduced and the thin basement membrane underlying the epithelium becomes continuous with the cuticular wall of the tubule leading into the bursa seminalis.

A flattened muscular ovovitelline duct (ductus communis) arises ventrally near the anterior end of the seminal receptacle and receives the ducts of the ovaries and vitellaria. It passes posterad through the mid-ventral parenchyma to about the level of the posterior end of the bursa seminalis and here enters the anterior end of the female antrum (Figs. 1, 2, 5). The ovovitelline duct is approximately 35 μ wide but is capable of considerable expansion to allow ova and yolk cells to pass into the uterus. Circular, oblique and longitudinal muscles are observable in contact with the thin basement membrane that underlies the lining epithelium; no fibrous sheath separates this duct from the cells of the mid-ventral parenchyma. Running parallel to the ovovitelline duct in the lateral parenchyma are paired accessory glands which enter the posterior part of the duct prior to its union with the female antrum (Figs. 1, 2, 5). Generally the cytoplasm of these gland cells stains evenly; however, in some cases the cells were observed to be filled with eosinophil granules.

The uterus arises ventrally from the anterior end of the female antrum. It extends anterad almost to the pharynx through the mid-ventral parenchyma and

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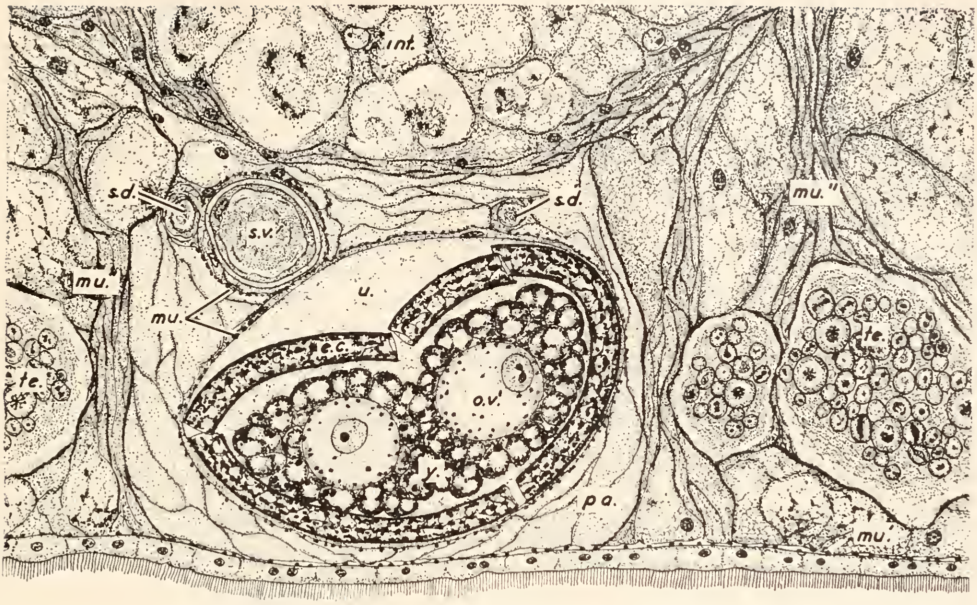
FIGURE 3. Transverse section through egg capsule and spermiducal vesicle (× 350).

FIGURE 4. Transverse section through entrance of ovary into seminal receptacle (× 500).

Abbreviations for Figures 3 and 4.

a.o.d.—accessory glands of ovovitelline duct, c.g.—cement glands, e.c.—egg capsule, int.—intestine, mu.—muscle sheath, mu′.—subepidermal muscles, mu″.—dorsoventral muscles of parenchyma, ov.—ovary, ov′.—ovum, p′.—base of penis, pa.—mid-ventral parenchyma, s.d.—sperm duct, s.r.—seminal receptacle, s.v.—spermiducal vesicle, te.—testis, u.—uterus, w.—whip of egg capsule, y.—yolk cells.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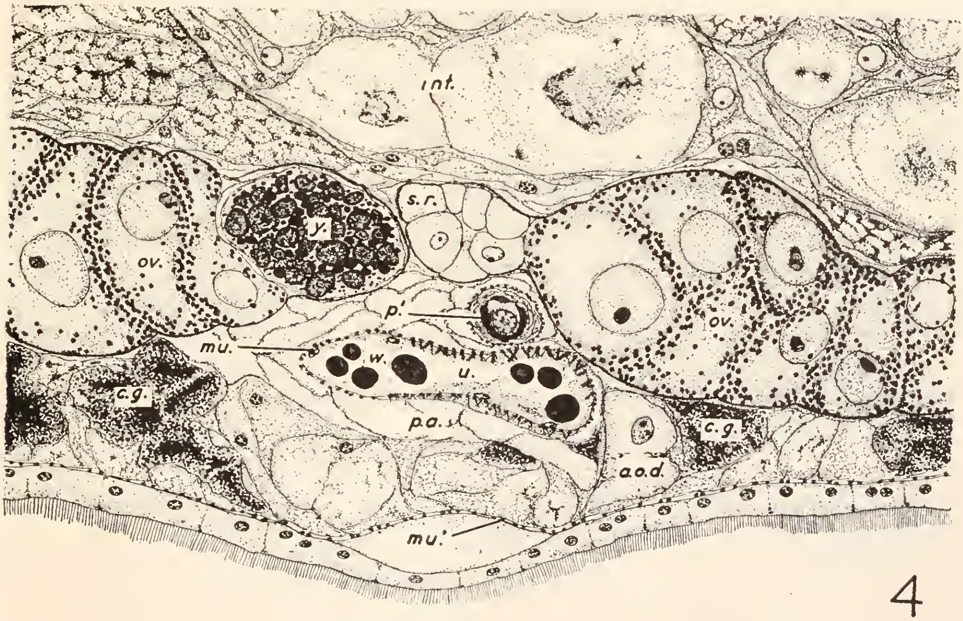
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Figures 3–4.

through its entire course lies very close to the ventral surface of the body (Figs. 1, 2, 4). The anterior end of the uterus is enlarged and encloses an amber-colored, oval egg capsule containing numerous yolk cells and from one to five spherical eggs (Figs. 1, 2, 3). The egg capsule is cuticular and possesses a whip-like prolongation that extends posterad through the entire length of the uterus and female antrum. Over most of its length the whip is about 10 μ thick. In the middle portion of the uterus the whip is often coiled back upon itself a number of times so that its total length may greatly exceed that of the uterus (Figs. 1, 2). The uterine wall is very similar in structure to the ovovitelline duct and is able to enlarge greatly to accommodate the egg capsule and the folded part of the egg whip (Figs. 3, 4).

The female antrum extends from the posterior ends of the uterus and ovovitelline duct to the common genital antrum (Figs. 1, 2). The walls are lined by columnar epithelial cells surrounded by a thin basement membrane and a muscular layer that is continuous with the fibers enclosing the uterus and ovovitelline duct. The lumen is about 12 μ in diameter and the posterior end of the egg whip, when present, almost completely fills this space (Figs. 5, 7). The ventrolateral spaces of the posterior third of the body contain numerous unicellular cement glands. The cytoplasm of these cells is generally uniformly filled with small granules that have a strong affinity for hematoxylin. Throughout the entire length of the female antrum many ducts from these glands enter the lateral walls (Figs. 1, 2, 5, 7). The secretions of the cement glands are believed to be associated with the attachment of the egg capsules to the substrate when expelled. Living animals compressed under a cover glass were occasionally observed at low magnification to undergo a series of rapid contractions which resulted in the extrusion of the egg capsule and whip. However, nothing is known about the normal deposition and attachment of the capsules, nor are other details of the life cycle understood.

The common genital antrum lies at the posterior end of the body. It is an elongated tube lined by flattened cells that appear to have cilia about 20 μ long which extend into the lumen (Figs. 1, 2, 7). A diffuse fibrous sheath separates this organ from the parenchyma. The common genital antrum receives the terminal ducts of both male and female reproductive systems: the bursal canal arises from it as a dorsal diverticulum; the male antrum enclosing the penis stylet is given off as a mesial evagination; and the female antrum enters it ventrally. The common genital pore opens on the ventral surface at the posterior end of the body. At this point

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FIGURE 5. Transverse section through bursa seminalis and bursal valve (× 350).

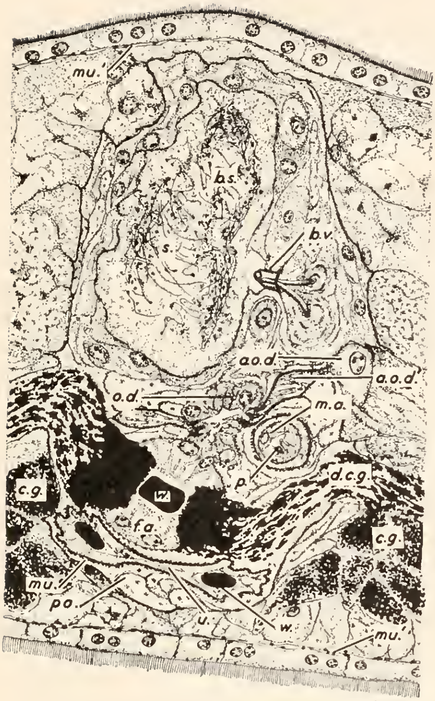
FIGURE 6. Bursal valve (× 1,050).

FIGURE 7. Transverse section through the entrance of female antrum and male antrum into the common genital antrum (× 350).

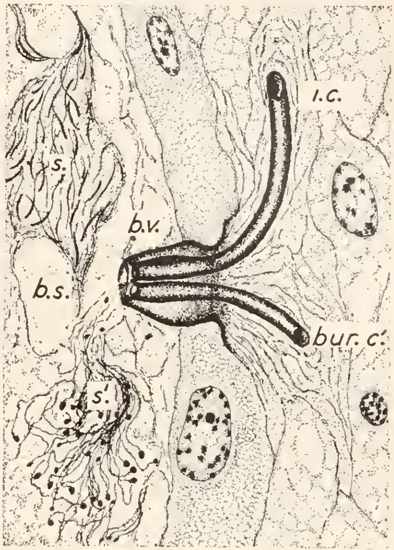
FIGURE 8. Transverse section through pharynx (× 200).

Abbreviations for Figures 5 through 8.

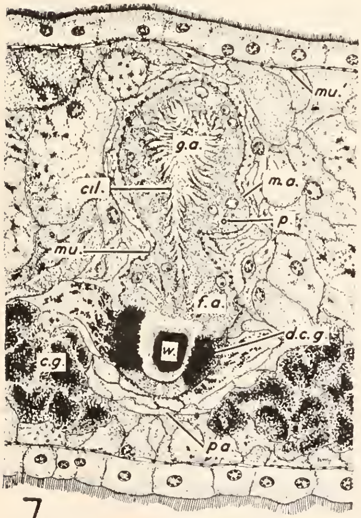
a.o.d.—accessory glands of ovovitelline duct, a.o.d'.—ducts of accessory glands of ovovitelline duct, b.c.—buccal cavity, bur. c.—bursal canal, bur. c'.—cuticular end of bursal canal, b.s.—bursa seminalis, b.v.—bursal valve, cil.—cilia, c.g.—cement glands, d.c.g.—ducts of cement glands, f.a.—female antrum, g.a.—common genital antrum, int.—intestine, i.c.—insemination canal, l. int.—lumen of intestine, m.a.—male antrum, mu.—muscle sheath, mu'.—subepidermal muscles, mu''.—pharyngeal protractor muscles, n.—nerves, oe.—oesophagus, o.d.—ovovitelline duct, pa.—mid-ventral parenchyma, p.—penis, ph.—pharynx, ph. g.—pharyngeal glands, s.—spermatozoa, s'.—degenerating spermatozoa, u.—uterus, w.—whip of egg capsule.


mu.
b.s.
s.
b.v.
a.o.d.
a.o.d.
o.d.
m.a.
p.
d.c.g.
c.g.
w.
f.a.
c.g.
mu.'
po.
u.
w.
mu.'

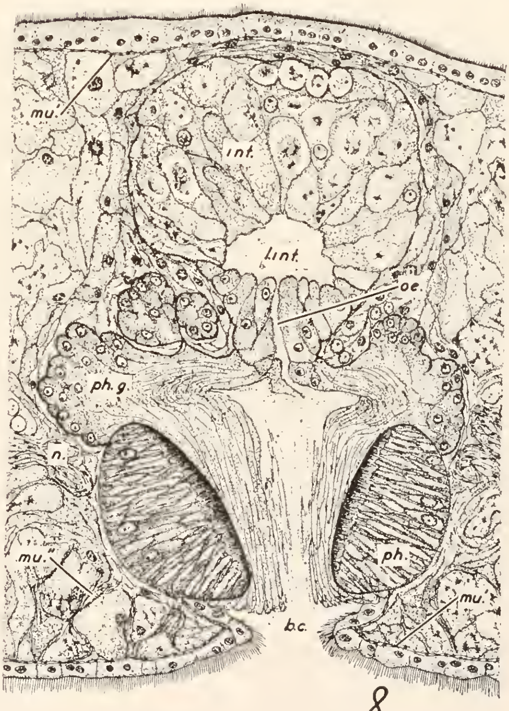
5


i.c.
s.
b.v.
b.s.
bur.c.'
s.'

6


mu.'
g.a.
cil.
m.a.
p.
mu.
f.a.
d.c.g.
c.g.
w.
p.a.

7


mu.'
int.
l.int.
oe.
ph.g.
n.
mu.''
ph.
mu.'
b.c.

8

FIGURES 5–8.

the ciliated ventral epithelium is invaginated and forms a short bulb-like canal which meets an outpocketing of the common genital antrum. Sphincters encircle both ends of this canal and regulate the size of the pore.

DISCUSSION

Comparison of genera

Although the parasite described here is similar in many respects to all genera in the family Umagillidae, there are certain structural characteristics that do not correspond to those of any previously reported genus of this family. Therefore, it is considered necessary to establish a new genus to be designated by the name *Syndisyrinx.* This name is intended to describe the complex bursal valve which is not present in any other genus of the family. The specific name, *Syn. franciscanus,* is given to designate the host, *Strongylocentrotus franciscanus,* in which it was first found.

For the sake of uniformity in the following comparison of genera of the family Umagillidae, the morphological nomenclature used by the various authors in their original descriptions of genera and species has been altered to conform with the terminology employed in the preceding analysis of *Syndisyrinx.*

In addition to the fact that both *Syndisyrinx* and *Syndesmis* are found in the intestine of echinoids, the morphological characteristics of *Syndisyrinx* indicate a closer relationship to *Syndesmis* than to the other genera of the family. The location and appearance of important organs, *viz.,* muscular pharynx, lobed testes, small spermiducal vesicle, muscular common sperm duct, ramified vitellaria, dichotomously branched ovaries, elongated uterus and egg capsule with whip, are very similar in *Syndesmis* and *Syndisyrinx* and strongly suggest a close relationship between these two genera. *Syndisyrinx* differs from *Syndesmis* chiefly in the structure and relationships of the bursa seminalis and seminal receptacle. In *Syndesmis* a single vesicle is present for the reception of sperm and cuticular structures such as the parts which make up the bursal valve of *Syndisyrinx* are lacking. In addition to these differences, the stucture of the penis is markedly dissimilar in these two forms. The penis of *Syndisyrinx* is a cuticular hollow stylet attached only at the base, whereas the copulatory organ of *Syndesmis* is a muscular eversible tube with a cuticular lining (Russo, 1895; Fig. 16).

Structures corresponding to the cuticular canals in the bursal valve of *Syndisyrinx* are found in *Anoplodiera voluta, Wahlia macrostylifera,* and *Desmote vorax.* In *A. voluta* the relationships of the two cuticular canals to the bursa seminalis, as described by Westblad (1930), are very similar to the arrangement of these structures in *Syndisyrinx.* However, the cuticular sheath that surrounds the entrance of these ducts into the bursa is lacking in *A. voluta.* There do not appear to be grounds for concluding that *Syndisyrinx* and *Anoplodiera* are closely related since the appearance and location of the testes and vitellaria, the presence of a single ovary, and the absence of a female antrum connecting the ovovitelline duct and uterus to the common genital antrum in *A. voluta* differ strikingly from the arrangement found in *Syndisyrinx.*

In *W. macrostylifera,* described by Westblad (1930), and *D. vorax,* according to Beklemischev (1916), the proximal end of the bursal canal is cuticular but an insemination canal is lacking. In other respects *W. macrostylifera* differs from

Syndisyrinx chiefly in regard to the morphology of the male reproductive system. The penis stylet is greatly elongated, and paired sperm ducts arising from compact testes unite and communicate by means of a single duct with the large spermiducal vesicle situated anterior to the pharynx. Many points of difference are likewise found by comparing the morphology of *Syndisyrinx* and *Desmote*. The most evident of these are the bipartite gut and the presence of two genital pores, the anterior pore by which the uterus opens to the exterior and the posterior pore which serves for copulation in *D. vorax*.

The other genera of the family lack cuticular parts in the copulatory complex comparable to those in the bursal valve of *Syndisyrinx* and to a greater or less degree exhibit dissimilarities in the location, distribution, number, arrangement and relationships of organs in the body. In these genera the most conspicuous differences with respect to *Syndisyrinx* are: the single ovary and absence of a cuticular copulatory stylet in the genus *Anoplodium;* the unbranched ovaries and double-walled cuticular penis stylet in the genus *Umagilla;* the absence of a cuticular penis and the general arrangement of testes and vitellaria in the genus *Xenometra;* and the single testis in the genus *Collastoma*. A manuscript is in preparation which will deal at greater length with the structural relationships of these forms.

Bursal valve

There is a superficial similarity between the bursal valve of *Syndisyrinx* and the cuticular nozzle-like mouthpieces of acoels. In the acoel, *Amphichoerus,* described by Graff (1891), and many allied forms, one end of the mouthpiece is generally connected to a vesicular sac or bursa filled with sperm; the other end is directed toward the ovary. L. H. Hyman (1937) points out that the function of these mouthpieces is apparently to direct sperm toward the ova to help insure fertilization. This function can hardly be ascribed to the insemination canals of *Anoplodiera* and *Syndisyrinx* which conduct sperm from the bursa seminalis to the seminal receptacle and not directly to the ova; nor does it seem probable that the insemination canals of Umagillidae are homologous to these mouthpieces. Noncuticular ducts connect the bursa seminalis to the seminal receptacle and bursal canal in most genera of Umagillidae, which suggests that cuticular structures are probably of relatively recent rather than primitive origin. In an analysis of the existing genera, Wahl (1910b) presents evidence which leads him to conclude that *Umagilla* is the most primitive and least modified genus of the family. If one accepts this view, it lends support to the opinion expressed above, inasmuch as *Umagilla* lacks any cuticular structures that might be considered homologous to the bursal valve. It is possible that the absence of cuticular parts in some of the species is due to a greater degree of simplification associated with a parasitic existence. However, there is no direct evidence for this supposition, since in the most closely related free-living families, Graffillidae and Dalyelliidae, cuticular structures such as these are not found. This suggests that these tubules have arisen independently, and until additional information is available, the insemination canals and bursal canals of Umagillidae should not be considered as mouthpieces in a true sense.

Although copulation has not been observed in *Syndisyrinx,* it is believed that the sperm of one animal are injected by means of the protrusible penis into the bursal canal of another. Before fertilization can take place, sperm must migrate from the bursal canal through its narrow proximal end into the bursa seminalis, there remaining until able to find their way through the insemination canal into the seminal receptacle. Evidently many sperm are unable to accomplish this migration and degenerate in the lumen of the bursa seminalis. Sperm that do reach the seminal receptacle must then pass through the constricted anterior part of this organ to fertilize the mature ova that enter the ovovitelline duct at the anterior end of the seminal receptacle.

It is difficult to explain any selective advantage for the presence of the fine canals that make up the bursal valve of *Syndisyrinx*. It was thought at first to be a mechanism for the prevention of polyspermy. However, this explanation is negated by the presence of large masses of spermatozoa in the seminal receptacle. The simplest explanation for the presence of these ducts is that they act as valves which regulate the number of spermatozoa entering the bursa seminalis and seminal receptacle. If this interpretation is correct, it is probable that the function of the bursal valve is to insure a necessary aging of the sperm in the bursa before fertilization. The cuticular walls are necessary to prevent the collapse of these narrow tubes. It is evident that the bursal valve restricts the free passage of sperm from the bursa seminalis and therefore as the result of a single copulation, a continuous supply of sperm may be maintained over a long period of time.

SUMMARY

After completing a histological study of an endoparasitic rhabdocoel from the Pacific Coast sea urchin, *Strongylocentrotus franciscanus,* the following conclusions have been reached:

1. This parasite belongs to the rhabdocoel family Umagillidae but differs in certain characteristics from the eight known genera of the family.
2. The distinguishing characteristics are a single intestine, paired and lobed ovaries and testes, a tubular single-walled cuticular penis stylet, and cuticular ducts connecting the bursa seminalis to the bursal canal and seminal receptacle.
3. A characteristic structure typical of this parasite and not present in other genera of the family is the bursal valve composed of two cuticular tubes, the insemination canal and proximal end of the bursal canal, which enter the bursa seminalis through a cuticular cup-like sheath.
4. The parasite here described is given the name *Syndisyrinx franciscanus, gen. et sp. nov.*

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A QUANTITATIVE STUDY OF THE RELATIONSHIP BETWEEN THE ACTIVITY AND OXYGEN CONSUMPTION OF THE GOLDFISH, AND ITS APPLICATION TO THE MEASUREMENT OF RESPIRATORY METABOLISM IN FISHES

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INTRODUCTION

The fact that fish consume more oxygen when active than when quiescent has been observed by many investigators (Krogh, 1916; Bowen, 1932; Clausen, 1933, 1936; Wells, 1935; Schlaifer, 1938; Smith and Matthews, 1942), but apparently no attempt has been made to determine the exact relationship between oxygen consumption and activity in fishes. It is the purpose of this paper to present data which are believed to provide an objective and quantitative basis for the relationship between activity and oxygen consumption in the goldfish, and to describe a method for making the necessary measurements. The method is based on the use of a recording activity detector (Spoor, 1941) combined with a continuous flow system for measuring oxygen consumption.

The lack of definite information on the activity of fish under experimental conditions has been one of the chief sources of difficulty in work on the respiratory metabolism of fishes, and attention has been called to the need for an experimental method which would make it possible to distinguish between "standard metabolism" and the increased metabolism due to muscular movements (Wells, 1935). In view of the fact that the oxygen consumption is affected by changes in the basal metabolic rate as well as by changes in activity, the importance of such a method is apparent. The method employed in the present work seems to meet this need, inasmuch as the state of activity is recorded continuously and periods of inactivity can be selected for measuring basal oxygen consumption.

Szymanski (1914) and Spencer (1939), using other types of activity detectors, have reported that goldfish show considerable individual variation in activity and that the activity pattern is affected by light. Spencer (1939) also found activity to be influenced by food. Knowledge of the behavior of the fish under the experimental conditions is of importance in the collection of data on oxygen consumption in the method to be described, as well as in the interpretation of these data. For this reason further observations on the patterns and rates of activity and on the effects of food, light and disturbances are included in the present paper.

THE ACTIVITY OF THE GOLDFISH UNDER EXPERIMENTAL CONDITIONS

Method

Several dozen goldfish (*Carassius auratus*) ranging between 24 and 96 grams in weight were selected at random from a stock obtained from a local goldfish farm and studied individually in experimental chambers, each of which was equipped with a recording activity detector. The experimental chambers were set up in a ground floor aquarium room which was seldom entered except for the purposes of this study, so that the fish could be left for long periods with relatively little disturbance. The recording apparatus was kept in another room. Records of the activity of each fish were started shortly after its introduction into a chamber and continued for periods ranging from a few days to many months in length, during which the patterns and rates of activity and the effects of food, light and disturbances upon them were studied. With a few exceptions, oxygen consumption was not measured in this series of observations.

The experimental chamber (Fig. 1) consisted of a one-gallon brown glazed crock fitted with a galvanized iron wire screen of ½ inch mesh to prevent the fish from reaching the surface of the water. A glass tube about 3 cm. in diameter was fitted into an opening in the center of the screen so that it extended 3.5 cm. above and 3 cm. below the screen; its purpose will be considered in a later section. The surface of the water stood about 3 mm. above the screen, the total volume to this level being 2,600 cc. Water entered the chamber from a constant level reservoir through 8 mm. glass tubing and left by way of a siphon of 8 mm. glass tubing which dipped into a constant level drain, the rate of flow (between 70 and 100 cc. a minute) being regulated by means of a glass stopcock in the inlet. The intake of the siphon was placed about 5 cm. above the bottom of the chamber, so that feces and other debris that fell to the bottom did not enter the siphon until they had been broken into small pieces in the course of their passage upward to the intake. The chamber was practically self cleaning under these conditions, the flow of the water and the movements of the fish being sufficient to move debris into the siphon. The fish

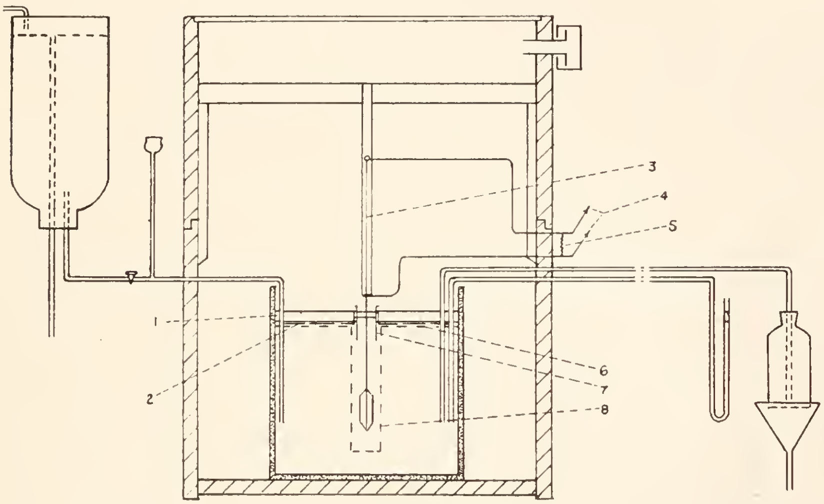

Figure 1. Diagram of apparatus for measuring oxygen consumption and activity. (1) paraffin oil (this was omitted when activity alone was being measured), (2) glass plates, (3) No. 44 copper wire, (4) to sensitive relay, (5) resistor, (6) wire screen, (7) glass tube, (8) wire frame protecting paddle. Explanation in text.

could therefore be maintained in the chamber for months without cleaning. A thistle tube entering the inlet provided for the introduction of food, being closed off at all other times. The water supply consisted of tap water passed through an activated charcoal filter, brought to the desired temperature and aerated until it approached equilibrium with the atmosphere. Most of the observations were made at temperatures between 20 and 24° C. The fish seldom extracted more than one-third of the oxygen from the water at the rates of flow employed, and they usually took less than this. In view of the findings of Crozier and Stier (1925), Toryu (1927), and Schlaifer (1938), it seems unlikely that behavior was influenced by the oxygen tension of the water.

The chamber was enclosed in a wooden case to minimize disturbances and to make it possible to control the light. The top of the case was fitted with a pane of glass for natural illumination, and with a wooden cover when either complete darkness or constant light was desired. A ventilator in the side of the case, with baffles to prevent light from entering, permitted some circulation of the air. The water inlet, outlet siphon, and a tube leading to a U tube indicating the water level in the chamber passed through the wall; a coat of black paint over each tube prevented light from entering the chamber through these openings.

The detector consisted of a light-weight aluminum paddle suspended in the water in the experimental chamber by a fine copper wire in such a way that a silver rod at the top of the paddle shaft passed through a small hole in a fixed silver plate. Water currents set up by the movements of the fish moved the paddle, causing the rod to make and break contact with the sides of the hole and thus to activate a sensitive relay. This relay operated the recording apparatus. The blade of the paddle consisted of aluminum foil (5 cm. long and 2.5 cm. wide) with the corners bent in at right angles so that the water currents struck a flat surface regardless of their points of origin. The shaft (10 cm. long) consisted of no. 22 aluminum wire cemented to the blade and imbedded at its upper end (7 cm. above the blade) in a bakelite insulating rod (2 cm. long and 0.2 cm. in diameter) in the upper end of which the silver rod (1 cm. long and about 0.04 cm. in diameter) was imbedded. This silver rod was soldered to a 14.5 cm. length of no. 44 enameled copper wire held in an insulated binding post attached to a wooden supporting shaft. A wooden bracket rising from the case supported this shaft in a vertical position so that the paddle hung in the water through the glass tube in the center of the screen. A cylindrical frame of galvanized iron wire protected the paddle from contact with the fish. A small lead weight (about 0.1 gm.) clamped to the paddle shaft below the bakelite helped to bring the paddle back to the resting position after displacement by the water currents. The silver plate (about 0.5 cm. square) was attached to the supporting shaft and held in a horizontal position about 6 cm. above the screen. The hole in the plate was between 0.08 and 0.1 cm. in diameter. The current to operate the sensitive relay was supplied by a 6 volt storage battery; the coil of the relay had a resistance of 1,000 ohms. A 5,000 ohm resistor across the detector contacts prevented sparking and welding without causing an observable reduction in the sensitivity of the detector. The plate was kept warm by means of a small insulated heating coil in order to prevent water from condensing upon it from the humid atmosphere above the chamber.

The sensitivity of the detector could be controlled somewhat by adjusting the position of the silver rod with respect to the sides of the hole in the plate. The normal movements of the operculum and the position-maintaining fin movements of a quiescent 25- to 30-gram goldfish were usually sufficient to move the paddle slightly, and when the rod was close to the plate these movements were recorded. For the observations to be described, however, the rod was centered in the hole so that the ordinary respiratory movements did not move the paddle enough to make contact, these movements being considered as among the basal functions of the fish. Vigorous respiratory movements and any movement that resulted in a change in the position of the fish moved the paddle enough to make and break contact, slow swimming movements causing few, and vigorous activity causing many impulses to be recorded. At the flow rates used in these experiments the flow of water through the chamber did not move the paddle.

The sensitive relay activated a counter which in turn caused signal magnets to record every tenth and hundredth impulse on a long paper kymograph moving about 30 mm. an hour. The frequency of the impulses was such (ranging up to 6,000 an hour) that they usually could not be counted when recorded individually. The counter was capable of following and recording at least 10 impulses a second. Time was recorded in hours beneath the activity record.

Patterns and rates of activity

In agreement with the results of Szymanski (1914) and Spencer (1939), the goldfish used in this study proved to be quite variable in their patterns and rates of activity, even when they were maintained under almost identical conditions of light, feeding, temperature, water supply and disturbance. Three general types of behavior appeared when the fish were kept under natural conditions of light: (1) arhythmic activity, in which no relation to day or night could be detected; (2) rhythmic activity, in which the fish were active by day and quiescent at night; (3) rhythmic activity, in which they were quiescent by day and active at night.

Fish showing the first, arhythmic, type of behavior were extremely variable. A few were vigorously active day and night for periods as long as ten days, others were moderately active throughout the 24-hour period for weeks at a time, and still others remained practically inert for similar periods. Some of these arhythmic fish, particularly those in the last group, showed irregular bursts of activity now and then, with no apparent relation to the time of day, feeding, or disturbance.

An example of the second type of behavior, diurnal activity and nocturnal quiescence, is shown in Figure 2, which is based on the number of impulses recorded by a 35-gram male goldfish during each hour between 1 P.M. January 11 and 1 P.M. January 13, 1946. The fish was fed at 11:05 A.M. on January 12, otherwise the room was not entered between 2:45 P.M. January 11 and 7:15 P.M. January 13. Aside from the feeding, the effects of which are discussed below, light was the only known variable, the temperature, rate of flow and aeration of the water being the same at the end as at the beginning of the period. Most of the fish showing rhythmic changes in activity followed patterns of this type, although the active phase varied considerably, sometimes being interrupted by several hours of quiescence during the day, sometimes beginning later in the day, and occasionally continuing well into the night. The most constant period of quiescence occurred between midnight and 4 A.M., which is in agreement with Spencer's (1939) observations.

The third type of behavior, diurnal quiescence and nocturnal activity, was found less frequently than the second, although it was not uncommon. An example is shown in Figure 3, which is based on records of the activity of a 32-gram male goldfish between 1 P.M. July 3 and 1 P.M. July 5, 1945. The room was not entered between 4 P.M. July 3 and 8 A.M. July 5, and aside from the daily changes in light the environmental conditions apparently remained constant throughout the period.

The patterns of rhythmic fish did not seem to be fixed, however, even when the environmental conditions remained unchanged. After several weeks of rhythmic behavior the fish frequently became arhythmic for several weeks or months, occasionally becoming rhythmic again in the course of extended periods of observation. This suggests that those fish which did not show daily activity rhythms under the experimental conditions may have done so eventually had they been studied for longer periods, and that by chance the observations were made during arhythmic periods.

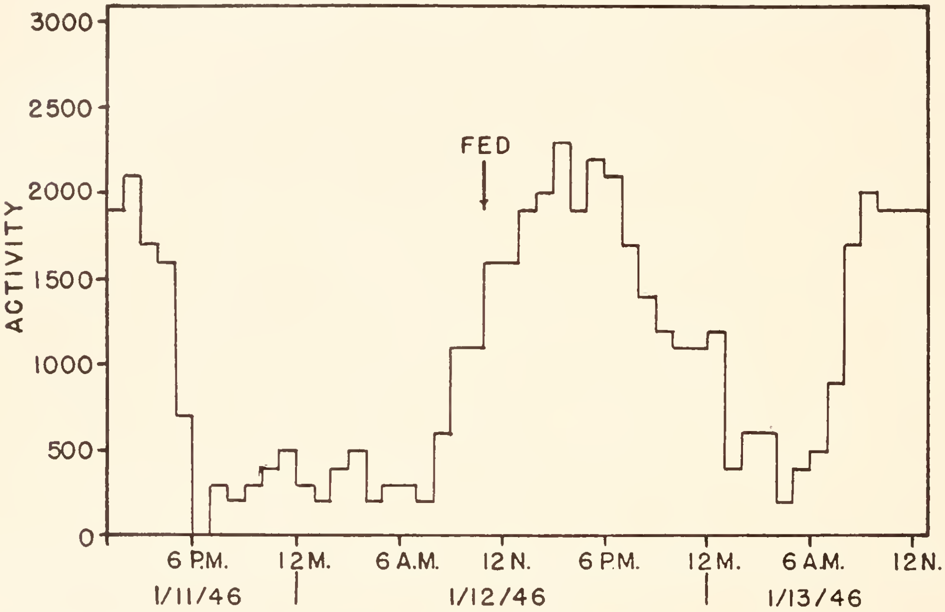

FIGURE 2. Activity pattern of 35-gram male goldfish between 1 P.M. January 11 and 1 P.M. January 13, 1946. Activity is expressed as number of impulses recorded each hour. Temperature 21.5° C. Fed at 11:05 A.M. January 12.

Activity and food

The fish were fed rolled oats, commercial fish foods, shredded shrimp, ground liver or chopped earthworms about three times a week, usually 0.5 to 1 gram at each feeding. The effects of daily feeding, larger amounts of food and starvation were also studied. Under the conditions of the experiments the type of food given had no consistent effects upon activity, but the quantity of food had pronounced effects, particularly on the total amount of activity. A well fed fish was usually sufficiently active that the number of impulses recorded in the course of a 24-hour period averaged between 500 and 1,500 an hour, and averages in excess of 2,500 impulses an hour were not uncommon. Starvation caused this rate to decrease markedly, sometimes to fewer than 100 impulses an hour, although as a rule the lowest rates did not appear until the fish had been starved for a week or so. No fish was observed to become completely inactive for periods of more than an hour or two, however, even when starved for two weeks. The effects of feeding after a period of starvation were striking, activity increasing to normal "well fed" rates within a few minutes. Doubtless the swimming movements associated with feeding accounted for some of the activity recorded following the introduction of food, but it seems that the nutritional state also affected the amount of activity. Food given in amounts of one gram or less was usually consumed within three to six hours, but the fish remained active (in accordance with their activity patterns) for from several days to a week after they had been fed. Similarly, Spencer (1939) found the goldfish to maintain a high rate of activity for several hours after feeding, although in his experiments the food was usually consumed within 15 minutes or less.

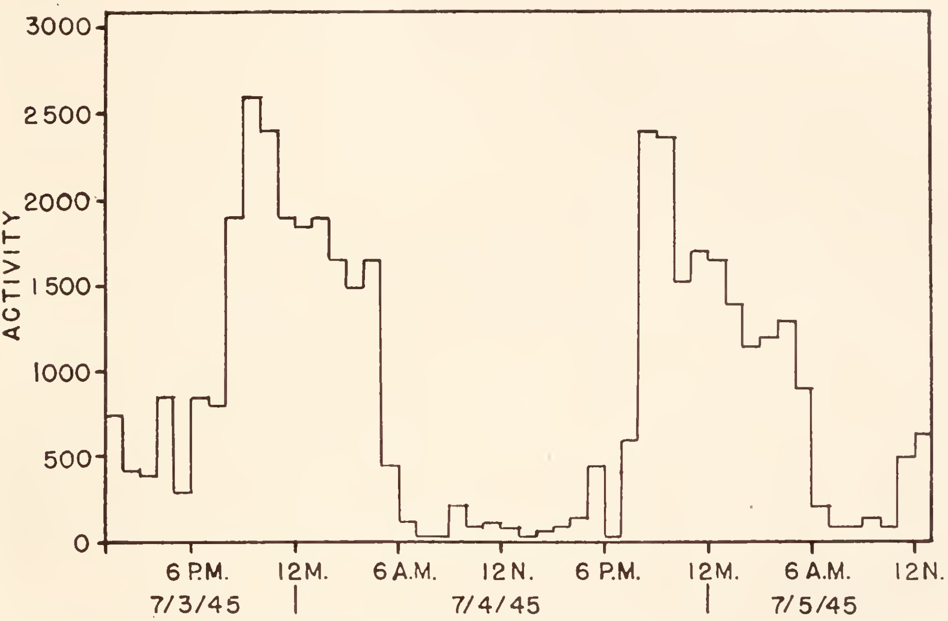

FIGURE 3. Activity pattern of 32-gram male goldfish between 1 P.M. July 3 and 1 P.M. July 5, 1945. Units as in Figure 2. Temperature 23.5° C.

The effects of feeding upon activity rhythms were not studied in detail, but the available data bearing on this question indicate that although the rhythms appearing under natural conditions of light were frequently modified by the quantity of food and the time of feeding, they were not causally related to food. Feeding modified the activity patterns of some fish for part or all of the subsequent 24-hour period, usually by prolonging the active phase. A response of this type may be seen in Figure 2. The fish was fed 0.5 gm. of rolled oats at 11:05 A.M. on January 12 (the previous feeding being on January 9); it will be noted that the activity level remained relatively high for a much longer period on the night of January 12 than on the preceding night. On the other hand some fish showed no change in activity in response to feeding, provided of course that they had not been starved. Variations in the quantity of food and in the time and frequency of feeding did not seem to have permanent effects on the activity rhythms, and feeding at the same time each day did not cause arhythmic fish to become rhythmic.

Activity and light

The goldfish did not seem to be much affected by changes in light intensity while they were not following daily activity rhythms, but they were usually quite responsive to light during their periods of rhythmic behavior. In fact, when the fish were well fed and undisturbed the activity rhythms seemed to be closely related to the daily changes in natural light, as Szymanski (1914) has reported previously. This view is supported by several observations in addition to the fact that the active and quiescent phases of the cycles usually coincided with day and night. Periods of nocturnal activity and diurnal quiescence were shown by the 32-gram male goldfish mentioned above in July and December of 1945. Although the water temperature and other factors except light were the same during both periods, the nocturnal phase of activity usually began earlier in the evening (between 5 and 6 P.M.) and ended later in the morning (between 7 and 8 A.M.) in December than in July, when it usually began between 7:30 and 8:30 P.M. and ended between 5 and 6 A.M. This suggests of course that the nocturnal phase of activity was limited by the setting and rising of the sun. This fish also responded readily to experimental changes in light intensity, particularly during the day, when darkening the chamber caused its activity to increase to levels usually reached only at night. Records were also obtained in which diurnally active and nocturnally quiescent fish remained active on nights when bright moonlight entered the room in which they were kept. Spencer (1939) found that the regular diurnal rhythm of the goldfish could be obliterated by covering the tank by day and lighting artificially at night. This procedure was accompanied by night feeding, however, so that the change in activity may not be attributed solely to the reversed lighting.

On the basis of these observations attempts were made to maintain goldfish at definite rates of activity by exposing them to continuous dim light and to continuous darkness for periods lasting as long as three weeks, but without success. The fish did not maintain constant rates of activity under either condition, but continued to alternate periods of increased activity with periods of relative quiescence. In order to maintain a low rate of activity it was necessary to starve the fish for about a week, the relationship between nutritional state and amount of activity being similar to that described in the preceding section.

Activity and disturbance

The goldfish proved to be extremely sensitive to disturbances. Noise, slight changes in the water level, sudden lights, the mere presence of the observer in the room, or such minor disturbances as the quiet opening and closing of the door to the room usually caused a change in the rate of activity. Fish that had been active before the disturbance almost invariably became less active, sometimes practically motionless, while quiescent fish frequently, although less consistently, became active when disturbed. Whichever the response, the original state of activity was usually resumed within a few minutes after the disturbance had ceased. The degree of response seemed to be related to the amount of disturbance, for when the observer moved slowly and quietly the change in activity was usually less pronounced, and recovery more rapid, than after ordinary passage through the room or adjustment of the apparatus. The effects of disturbances upon the activity of an otherwise quiescent fish may be seen in Figure 3. The room was entered several times in the course of the afternoon of July 3 and on July 5, although the experimental chamber was not approached and the fish could not see the cause of the disturbance. It is obvious that the rates of activity were higher than at corresponding hours on July 4, when the room was not entered. Such sensitivity has been observed in other species of fish by Clausen (1934), who found that a shadow passing over the aquarium caused increases in the body temperatures of perch and members of the sunfish group.

The Relationship Between Activity and Oxygen Consumption

Method

The activity and corresponding oxygen consumption of individual goldfish were measured in observation periods ranging in length from 11 to 210 minutes. Activity was measured in terms of the number of impulses recorded in a given period, and the amount of oxygen consumed by the fish in that period was determined by means of a continuous flow system. A control chamber similar to the experimental and housed in the same case was supplied with a continuous stream of water from the reservoir supplying the fish. The water in each chamber was covered with a layer of heavy paraffin oil 2.5 cm. thick to retard the diffusion of oxygen from the air, and a sample of the effluent from each chamber was analyzed for oxygen by the Winkler method at the beginning and end of each period. The samples were collected in narrow necked glass stoppered bottles of about 270 cc. capacity arranged to serve as constant level drains (Fig. 1). Each line was arranged so that the water passed through the outlet siphon to the bottom of the sampling bottle and overflowed into a funnel so that it could be collected for flow rate determinations. Although the rates of flow ranged from 70 to 100 cc. a minute in the course of the study, the rate for any one day's series of samples was held practically constant. Due care was taken to prevent the diffusion of oxygen into the samples and to obtain representative samples from experimental and control lines. Samples that were contaminated by particulate matter were discarded. The permanganate modification was used in most of the analyses, but was omitted during some of the shorter periods. The results obtained with and without the modification were quite similar, however, which was not unexpected in view of the fact that from four to six liters of water passed over the fish each hour.

The volume of water flowing through the system in the course of an observation period being known, together with the oxygen content of the water leaving the control and experimental chambers at the beginning and end of that period, the oxygen consumed by the fish could be calculated. The calculations took into account the change in the amount of oxygen in the constant volume of water in the chamber. The volume of water displaced by the fish was too small to affect the calculations.

The samples were collected with the foregoing observations on activity patterns and modifying factors in mind, the periods being timed to yield data at the activity rates desired, and the method of sampling being modified as necessary to minimize disturbance of the fish. In the latter connection the outlet tubes were lengthened so that samples were collected about 10 feet away from the chambers, and the room was not entered except for sampling and rate of flow determinations. Precautions were taken to prevent changes in the water level in the experimental chamber as there were indications that small changes in the level stimulated the fish. These precautions were necessary also because the volume of water in the chamber, as well as that flowing through it, entered into the calculations of oxygen consumption. Samples were discarded if subsequent examinations of the activity records showed that they had been collected while the fish was undergoing marked changes in activity as a result of disturbance or in accordance with an activity rhythm. This was necessary because although the activity record was instantaneous the change in the oxygen concentration of the samples tended to lag somewhat behind that in the chamber, the sample drawn at any instant representing the average of the water flowing into the bottle in the few minutes preceding its removal. The temperature of the water was recorded for each observation period in order to avoid discrepancies attributable to the effect of temperature on metabolic rate (Ege and Krogh, 1914).

Owing to its viscosity and the accumulation of emulsified oil and water at the oil-water interface, the layer of oil interfered with the movements of the paddle shaft. Its thickness was therefore reduced to 1 cm. within the central glass tube, thus permitting the paddle to move about as freely as with a water surface. This tube extended below the interface far enough to prevent the emulsion from accumulating around the paddle shaft. The oil within the tube had to be changed now and then, however, to remove the small amount of debris that entered it from beneath. The detector contacts and bakelite rod were cleaned every few days as a precaution against their becoming coated with oil, which seemed to spread slowly up the paddle shaft.

It was established by appropriate tests that the layer of paraffin oil was effective in preventing the diffusion of significant amounts of oxygen into the water from the atmosphere. In no test did the apparent leakage exceed the limits of error of the Winkler method itself (Allee and Oesting, 1934), and it was usually considerably less. The average apparent rate of change for the contents of the experimental chamber was 0.0015 cc. of oxygen a minute, which was so much smaller than the rate at which the fish consumed oxygen that even had the apparent change been real it would have had but little effect on the results. It should be mentioned in this connection that the oil layer was disturbed relatively little by the movements of the fish, inasmuch as the wire screen kept the fish out of the oil and glass plates resting on this screen lessened the churning effects of the water beneath it.

Results

Three goldfish were studied at several temperatures in a total of 104 observation periods. As the results on all three were much alike, data on but one of the fish, a 32-gram male on which over two-thirds of the measurements were made, are presented here.

The relationship between activity and oxygen consumption at temperatures between 23 and 25° C. is shown in Figure 4, in which oxygen consumption in cubic centimeters per minute is plotted against activity in impulses per minute. Fifty-nine observation periods are represented, each point corresponding to one period. The line merely indicates the trend, and has not been fitted to the data mathematically. As was to be expected, oxygen consumption and activity proved to be closely related, the relationship apparently being linear above the basal level of oxygen consumption. Although the values for oxygen consumption at any one rate of activity are seen to vary somewhat, the trend is clear cut: at high rates of activity the rate of oxygen consumption is correspondingly high; at low activity rates less oxygen is consumed. The discrepancies that do occur may well have been due to errors in measurement, rather than to a lack of correspondence between activity and oxygen consumption. In this connection the data on oxygen consumption follow those on activity quite closely when the comparison is restricted to one day's series of measurements, thus ruling out discrepancies attributable to slight differences in the adjustment of the detector contacts. Such a series is shown in Figure 5, which is based on data obtained with the same fish in a series of thirteen consecutive 15- to 25-minute periods at 22° C.

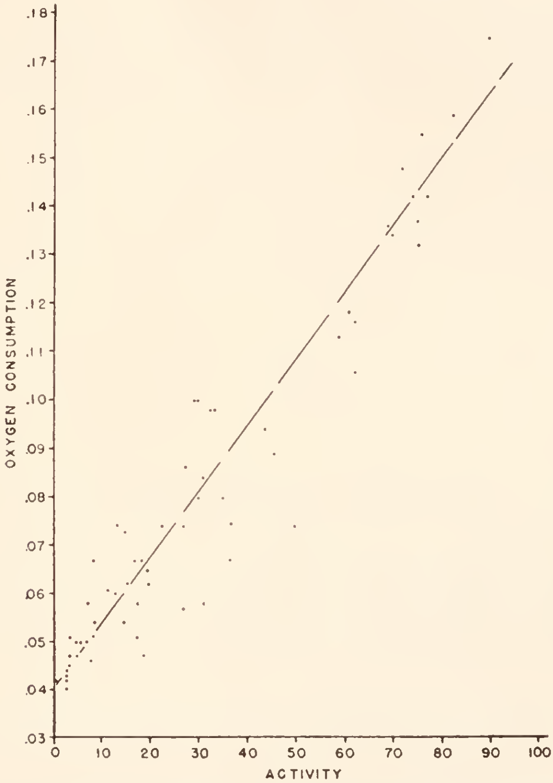

FIGURE 4. Activity and oxygen consumption of 32-gram male goldfish. Activity in impulses/minute; oxygen consumption in cubic centimeters/minute. Temperature 23 to 25° C.

According to the slope of the data shown in Figure 4, the basal oxygen consumption of this fish was in the vicinity of 0.040 cc. a minute, or 0.075 cc. per gram per hour.

DISCUSSION

The results of the present study have a bearing on the collection and interpretation of data on the respiratory metabolism of fishes, and in the light of these results the method described seems to offer a number of advantages not found in previous methods which have been employed for this purpose.

The advantages of the continuous flow method for measuring respiratory metabolism in fishes have been discussed by Keys (1930) and need not be reviewed here. In view of the relationship between oxygen consumption and activity, however, the observations on the effects of disturbances may be applied to the use of this method, inasmuch as the process of sampling may disturb the fish. Should a change in the rate of activity (and consequently of oxygen consumption) occur at the time of sampling, the sample would not be representative of the volume of water and unit of time to which it is related in the calculations. The resulting error could be of considerable importance, particularly in investigations in which the samples consisted of water flowing directly from the experimental chamber and overflowing through a sampling bottle. This source of error has been recognized of course, and in some investigations the experimental chamber has been covered in attempts to minimize stimulation of the fish. It seems very doubtful, however, whether covering a goldfish so that it cannot see the investigator is an adequate safeguard against disturbance. One advantage of using an activity detector in the continuous flow method then lies in the fact that any sudden change in activity occurring at the time of sampling can be detected, so that the reliability of the sample may be judged. Furthermore, the activity record can be used to test the effectiveness of the steps taken to avoid disturbance.

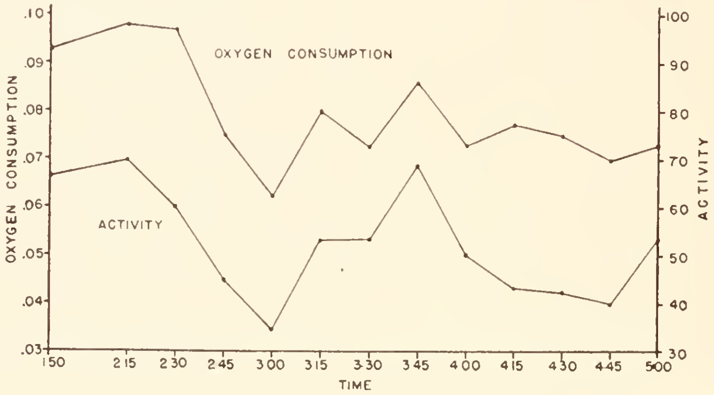

FIGURE 5. Activity and oxygen consumption of 32-gram male goldfish in each of thirteen consecutive observation periods between 1:35 P.M. and 5 P.M. November 13, 1945. Units as in Figure 4. Each point on the upper line represents the average rate of oxygen consumption for the 15- or 25-minute period preceding it. Each point on the lower line represents the average rate of activity for the corresponding period.

It is of course well known that fluctuations in activity during the test periods constitute a major obstacle to the correct interpretation of measurements of oxygen consumption, and numerous attempts to overcome this difficulty have been described (Ege and Krogh, 1914; Hall, 1929; Adkins, 1930; Keys, 1930; Wells, 1932, 1935; Clausen, 1933; Smith and Matthews, 1942). These measures include the use of narcotics, observing that the fish remains quiet, maintaining constant conditions of light, sampling at the same time each day, restricting the movements of the fish, and maintaining the fish in an experimental chamber until it appears to have come to rest or at any rate to have reached a steady state. Although such measures may permit the establishment of the reality of a change in oxygen consumption in connection with an experimental procedure, they do not appear to give a completely satisfactory basis for the interpretation of that change. The interpretation must be based on knowledge of the activity of the fish, inasmuch as oxygen consumption is affected by changes in the basal metabolic rate as well as by activity. The method employed must therefore be capable of supplying information on activity and oxygen consumption at the same time, so that the fraction of the respiratory exchange associated with basal metabolism may be distinguished from that due to muscular activity (Wells, 1935). None of the above methods seems to be adequate for this purpose.

Narcotics are of doubtful value in studies of this type, even for measuring basal metabolic rate alone (Adkins, 1930). Among other objections are indications that an important fraction of the metabolic functions of the fish may be suppressed to such an extent that the oxygen consumption falls below the basal level as it is generally understood (Keys and Wells, 1930). In fact, Ege and Krogh (1914) considered it necessary to use artificial respiration to insure the survival of their goldfish, the narcotic having interfered with normal respiratory movements. The other methods are open to criticism because they are based on the assumption, rather than the knowledge, that the fish is quiescent or at a constant level of activity under the conditions of the experiment. The results of the present work suggest that for the goldfish at any rate this assumption may be unwarranted. The fact that a goldfish is quiescent while it can be seen should not be taken as proof that it remains so while unobserved, and it does not seem justifiable to assume that constant environmental conditions mean constant rates of activity. So far as the goldfish is concerned, the individual variations in activity open to question the reliability of methods based on sampling at the same time each day, particularly if several fish are being compared. Confining the fish to a small respiration chamber to restrict its movements gives no assurance that it will remain quiescent or even at a constant rate of activity, and the fact that the oxygen consumption varies over a wide range in such chambers supports this objection. This method would seem to have a further disadvantage for measuring the basal metabolic rate in that a fish confined to a small tube must swim continuously, however slowly, in order to maintain its position in the current. The practice of leaving the fish in the respiratory chamber until its oxygen consumption has reached a relatively low and constant rate (Keys, 1930; Wells, 1935) is far superior to the earlier techniques, but it is limited in its application by the fact that it gives no information as to the amount of activity associated with the steady state.

The requirements of a satisfactory method appear to be met by combining an activity detector with the continuous flow system. The rate of activity can then be measured at the same time that oxygen consumption is determined, and the results interpreted accordingly. As the activity record is continuous, periods of quiescence can be selected for measuring basal oxygen consumption, so that it is not necessary to employ special techniques designed to control activity. In this connection, however, starvation may be used as a means of prolonging the quiescent state. A further advantage of the present method lies in the fact that the fish can be maintained in good health in the experimental chamber for months, so that measurements of its respiratory metabolism need not be obscured by the excitement and other effects of handling.

SUMMARY

1. Apparatus for making continuous records of the activity of isolated and undisturbed goldfish is described, together with a method for measuring oxygen consumption and activity simultaneously.

2. The goldfish were quite variable in their patterns and rates of activity under the experimental conditions. Some fish were diurnally active and nocturnally quiescent, others followed the opposite pattern and still others were arhythmic throughout the periods during which they were observed. Moreover, some fish showed both rhythmic and arhythmic states of activity when studied for periods extending over several weeks or months.

3. Food, light and minor disturbances had pronounced effects on the activity of the goldfish.

4. Simultaneous measurements of oxygen consumption and activity are presented which indicate that the two are closely related above the basal level of oxygen consumption.

5. The bearing of these observations on the collection and interpretation of data on the oxygen consumption of the goldfish and on the measurement of its basal metabolic rate is discussed, and certain advantages of the method are described.

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